

1 **BOTTOM-UP CONTROL OF PLANT DEFENSES AND CLIMATE ON**  
2 **GEOGRAPHIC VARIATION IN INSECT HERBIVORY ON WILD COTTON**  
3 *(Gossypium hirsutum)*

4  
5 LUIS ABDALA-ROBERTS<sup>1\*</sup>, TERESA QUIJANO-MEDINA<sup>1</sup>, XOAQUÍN MOREIRA<sup>2</sup>,  
6 CARLA VÁZQUEZ-GONZÁLEZ<sup>2</sup>, VÍCTOR PARRA-TABLA<sup>1</sup>, JORGE C. BERNY  
7 MIER Y TERÁN<sup>3</sup>, LUCA GRANDI<sup>4</sup>, GAÉTAN GLAUSER<sup>5</sup>, TED C. J. TURLINGS<sup>4</sup>,  
8 AND BETTY BENREY<sup>6</sup>

9  
10 <sup>1</sup>Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias,  
11 Universidad Autónoma de Yucatán, Apartado Postal 4-116, Itzimná, 97000 Mérida,  
12 Yucatán, Mexico

13 <sup>2</sup>Misión Biológica de Galicia (MBG-CSIC), Apdo. 28, 36080, Pontevedra, Spain

14 <sup>3</sup>Department of Plant Sciences, University of California-Davis, One Shields Avenue, Davis,  
15 California, USA 95616

16 <sup>4</sup>Fundamental and Applied Research in Chemical Ecology (FARCE Lab), Institute of  
17 Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel,  
18 Switzerland

19 <sup>5</sup>Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Rue Emile Argand  
20 11, 2000 Neuchâtel, Switzerland

21 <sup>6</sup>Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel,  
22 Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

23  
24 \*Corresponding author:

25 Luis Abdala-Roberts

26 Email: abdala.luis@yahoo.com

27 Phone Number: +52 999 9423206

28

29 Manuscript received\_\_\_\_\_; revision accepted\_\_\_\_\_;

30 **ABSTRACT**

31 *Premise of study:* It has long been recognized that the occurrence and impact of herbivory  
32 is shaped by bottom-up forces, primarily plant traits (e.g. defenses) as well as by abiotic  
33 factors. Addressing these concurrent effects under a spatial context has been useful in  
34 efforts to understand the mechanisms governing variation in plant-herbivore interactions.  
35 Still, few studies have evaluated the simultaneous influence of different sources of bottom-  
36 up variation on spatial variation in herbivory.

37 *Methods:* We tested to what extent plant chemical (phenolics, gossypol glands) and  
38 physical (pubescence) defensive traits and climatic factors are associated with variation in  
39 herbivory by leaf-chewing insects among populations of wild cotton (*Gossypium hirsutum*).

40 *Key results:* We found substantial variation among cotton populations in leaf defenses and  
41 insect leaf herbivory. Leaf pubescence (trichome density), but not gossypol gland density or  
42 phenolic content, was significantly negatively associated with herbivory by leaf-chewing  
43 insects. In addition, there were direct effects of climate on defenses and herbivory, with leaf  
44 pubescence increasing towards drier conditions and leaf damage increasing towards wetter  
45 and cooler conditions. There was no evidence, however, of indirect effects (via plant  
46 defenses) of climate on herbivory.

47 *Conclusions:* Spatial variation in insect herbivory on wild *G. hirsutum* appears to be  
48 predominantly driven by concurrent and independent influences of population variation in  
49 leaf pubescence and climatic factors.

50

51 **Keywords:** *herbivory, leaf pubescence, plant defenses, spatial variation, wild cotton.*

52

53

## 54 INTRODUCTION

55 Research has long recognized that herbivory by insects is strongly determined by bottom-  
56 up factors, such as plant defenses and abiotic variables (Rhoades, 1979; Agrawal 2011).  
57 The study of these factors under a geographic context (e.g. along ecological gradients) has  
58 been particularly useful to evaluate the mechanisms by which bottom-up forcing shapes  
59 plant-herbivore interactions (Gripengberg and Roslin, 2007; Hunter and Price, 1992; Abdala-  
60 Roberts and Mooney, 2015). Notably, studies along latitudinal or altitudinal gradients have  
61 commonly found higher levels of plant defenses and herbivory towards warmer and wetter  
62 climates present at lower latitudes and elevations (Fig. 1, arrow A; reviewed by Anstett et  
63 al., 2016; Moreira et al., 2018a). These patterns have been attributed to greater herbivore  
64 abundance and diversity under seasonally less variable, warmer and wetter climates,  
65 resulting in more intense plant-herbivore interactions and thus stronger selection on plant  
66 qualitative or quantitative defenses to more effectively reduce herbivory (Fig. 1, arrow B;  
67 Schemske et al., 2009; Zhang et al., 2016; but see Moles et al., 2011).

68 Abiotic factors vary substantially across space, shaping concomitant inter- and  
69 intra-specific plant variation in defensive traits and herbivory (Johnson and Rasmann, 2011;  
70 Pearse and Hipp, 2012; Hahn and Maron, 2016; Moreira et al., 2018a). For instance,  
71 climatic conditions may directly affect herbivore population size or behavior and thus  
72 herbivory rates (Fig. 1, arrow C). In addition, climatic factors may affect plant defenses,  
73 e.g. via allocation constraints; increased precipitation (and thereby higher resource  
74 availability) favors greater plant growth which then leads to lowered plant defences due to  
75 underlying growth-defense trade-offs (Fig. 1, arrow D; Resource Availability Hypothesis,  
76 Coley et al., 1985; Endara and Coley, 2011). By the same logic, increasing temperature  
77 could reduce growth and in turn favor increased defenses (Fig. 1, arrow D; warming could

78 also lower defences due to thermal stress reducing defence allocation). These climate-  
79 mediated effects on defenses may in turn indirectly (positively or negatively, depending on  
80 sign of direct effects of climatic variables on plant defenses) shape geographic variation in  
81 herbivory (Fig. 1, arrow E; e.g. Pratt et al., 2017; Moreira et al., 2018b). Although a  
82 number of studies have contributed to uncover these relationships, relatively few have  
83 simultaneously assessed the relative influences of variation in plant traits and abiotic factors  
84 on herbivory, thus limiting our understanding on the mechanisms underlying bottom-up  
85 control of spatial variation in herbivory.

86 Wild cotton, *Gossypium hirsutum* L., is a perennial plant that is naturally  
87 distributed along the northern and western coastal scrublands of the Yucatan Peninsula  
88 (D'Eeckenbrugge and Lacape, 2014). This species possesses physical (e.g. trichomes) and  
89 chemical (e.g. terpenoids, phenolics) defenses against herbivores (Abdala-Roberts et al.,  
90 2019), and experiences substantial variation in climatic conditions along its relatively  
91 narrow distribution range (e.g. > two-fold variation in mean annual precipitation along 2.5°  
92 latitude). Based on this, we sought to disentangle the associations between insect herbivory  
93 and plant defensive traits and climatic factors in this species. Our goals were to: (i) assess  
94 geographic variation in leaf defensive traits and insect leaf herbivory across populations of  
95 wild *G. hirsutum*; (ii) determine whether plant defensive traits are associated with (and  
96 presumably underlie) population variation in herbivory; and (iii) evaluate the effect of  
97 variation in climatic factors on population variation in cotton defensive traits and herbivory,  
98 and whether effects of climate on herbivory are mediated (indirectly) by abiotic controls on  
99 plant defensive traits. Although a large number of studies have tested for spatial variation  
100 (e.g. latitudinal, elevational) in herbivory or plant defenses, fewer have measured herbivory  
101 and defenses, and only a handful have combined data on herbivory, plant defenses, and

102 abiotic factors. In achieving the latter, this study provides a robust assessment of the  
103 independent contribution of multiple bottom-up factors concurrently shaping geographic  
104 variation in plant-herbivore interactions.

105

## 106 **MATERIAL AND METHODS**

107 **Study species** — *Gossypium hirsutum* is a perennial shrub that grows up to 2 m tall under  
108 natural conditions (Oosterhuis and Jernstedt, 1999). It is native to Central America, Mexico  
109 and the Caribbean Basin (Wendel et al., 1992; Oosterhuis and Jernstedt, 1999), and is  
110 thought to have originated in southeast Mexico (D'Eeckenbrugge and Lacape, 2014). Wild  
111 populations are common along the coasts of the Yucatan Peninsula (Mexico) growing in  
112 the coastal scrubland or sand dune vegetation (D'Eeckenbrugge and Lacape, 2014). In these  
113 habitats, wild *G. hirsutum* is attacked by a diverse community of insect herbivores, among  
114 which the most important are leaf chewers belonging to Orthoptera, Lepidoptera, and  
115 Coleoptera, whereas sap feeders (Hemiptera) are relatively less common (results from this  
116 study).

117       Much of the work on direct chemical defenses in *G. hirsutum* comes from studies  
118 with domesticated varieties (reviewed by Hagenbucher et al., 2013), whereas comparatively  
119 little research has focused on wild populations of this species (for work with other wild  
120 species of *Gossypium* see Rudgers et al., 2004). As direct chemical defenses, both wild and  
121 cultivated *G. hirsutum* plants produce pigment glands throughout their surface which  
122 contain gossypol and other related terpenoid aldehydes that have insecticidal effects  
123 (McAuslane et al., 1997; Rudgers et al., 2004; Stipanovic et al., 2006). Likewise, phenolic  
124 compounds have also been shown to provide direct resistance against insect herbivores in  
125 this species (Mansour et al., 1997; Nix et al., 2017; Abdala-Roberts et al., 2019). In

126 addition, wild *G. hirsutum* produces direct physical defenses such as non-glandular  
127 trichomes which are thought to play a role in biotic (as well as abiotic) resistance in this as  
128 well as other *Gossypium* species (Rudgers et al., 2004). On the other hand, *G. hirsutum* also  
129 resorts to indirect defense by producing extrafloral nectar and volatile compounds which  
130 attract ants and parasitoids, respectively and these traits have been argued to mediate  
131 indirect resistance in this species (McCall et al., 1994; Wäckers and Bezemer, 2003;  
132 Rudgers et al., 2004), though their defensive role in wild populations has not been yet  
133 evaluated.

134

135 **Population sampling** — In September 2017, we sampled 26 wild cotton populations  
136 located along the coast of Yucatan and Campeche (Fig. 2; Appendix A, *supplementary*  
137 *material*), spanning virtually all of its distribution range in SE Mexico (see  
138 D'Eeckenbrugge and Lacape, 2014). Despite its relatively narrow distribution range in the  
139 Yucatan Peninsula, abiotic conditions vary across the sampled sites. Specifically, mean  
140 annual precipitation varies up to 2.2-fold across the sampled sites, and although mean  
141 annual temperature varies considerably less (up to 1.3°C), mean maximum temperatures of  
142 the warmest month vary up to 3°C across sites (Appendix A). Distance between adjacent  
143 sites was at least 2 km and at each site we sampled 7-8 plants separated by at least 2 m.  
144 Plants were similar in size and ranged in height from ca. 1 to 1.5 m. We sampled plants in  
145 late September, which represents the last third of the wet season and is when insect  
146 herbivore abundances are highest and most of the insect leaf damage had already occurred.  
147 We measured plant cover for each individual by multiplying the length (longest distance  
148 from branch tip to tip) and width (perpendicular to former).

149 For each plant, we also visually estimated the percentage of area removed per leaf

150 by chewing insects by selecting a 1-m branch or two 0.5-m branches and using the  
151 following scale: 0%, 1-15%, 16-30%, 31-45%, 46-60%, and 61-75%. This resulted in ca.  
152 40-50 sampled leaves per plant. We used the midpoint of each category per leaf and  
153 averaged values across leaves to obtain a single (mean) value per plant for statistical  
154 analysis. Damage by leaf-chewing insects accounted for more than >90% of the recorded  
155 leaf herbivory; the remaining leaf damage was caused mostly by leaf miners (T. Quijano-  
156 Medina, pers. obs.). By sampling towards the end of the rainy season, our estimates of leaf  
157 herbivory represented a rough measure of cumulative leaf damage occurring during  
158 previous months and therefore of overall damage levels during the study growing season.  
159 After assessing leaf herbivory, we collected 6-8 undamaged leaves per plant for  
160 quantification of defensive traits, namely: density of gossypol glands, density of trichome  
161 needles (i.e. pubescence), and phenolic compounds (see ahead). Sampling undamaged  
162 leaves was aimed at measuring constitutive levels of these traits, though some degree of  
163 systemic induction has to be assumed. We counted the number of glands and trichomes on  
164 two 0.20 cm<sup>2</sup> disks obtained from each of two leaves using a stereoscopic microscope.  
165 Trichome counts were conducted on the adaxial surface whereas glands were counted on  
166 the abaxial leaf surfaces. Values per disk were extrapolated to 1 cm<sup>-2</sup> to obtain the number  
167 of trichomes or glands cm<sup>-2</sup>, and these values were then averaged across disks to generate a  
168 single value per plant for statistical analyses. Trichomes are needle-like and may show  
169 ramifications (multiple needles per structure). In most cases, individual trichomes had 1-3  
170 needles but in some cases they presented up to 6 needles (stellate morphology). We  
171 therefore counted the total number of needles rather than the number of trichomes as the  
172 former represented a better proxy of the amount of leaf pubescence. Hereafter we refer to  
173 trichome needle counts as leaf pubescence.

174 **Chemical analyses** — We collected 4-5 undamaged leaves from four of the experimental  
175 plants at each site for chemical analyses. Upon collection, leaves were stored in a cooler  
176 and then transported to the laboratory where they were immediately dried at 45°C. We  
177 extracted phenolic compounds using 20 mg of dry plant tissue (pool of leaves per  
178 individual) with 1 mL of 70% methanol in an ultrasonic bath for 15 min, followed by  
179 centrifugation (Moreira et al., 2014). We then transferred the extracts to chromatographic  
180 vials to perform phenolic profiling. Briefly, we used ultrahigh-pressure liquid  
181 chromatography-quadrupole-time-of-flight mass spectrometry (UHPLC-QTOF-MS) to  
182 detect, identify and quantify phenolic compounds. The separation was carried out on a 50 ×  
183 2.1 mm Acquity UPLC BEH C18 column (Waters, Milford, CT, USA) thermostated at  
184 25°C. Solvents were water + 0.05% vol. formic acid (A), and acetonitrile + 0.05% vol.  
185 formic acid (B). The gradient program was performed at a flow rate of 0.4 mL/min under  
186 the following conditions: 5-30% B for 6 min, 30-100% B for 2 min, holding at 100% B for  
187 2 min followed by re-equilibration at 5% B for 2 min with an injection volume of 2 µl. The  
188 QTOF-MS was operated in MS<sup>E</sup> negative mode over an m/z range of 85-1200 Da with the  
189 following parameters: capillary voltage at -2.5 kV, cone voltage -25 V, source temperature  
190 120°C, desolvation gas temperature 350°C, desolvation gas flow 800 L/hr. The instrument  
191 was internally calibrated by infusing a solution of leucine-enkephaline at 400 ng/mL at a  
192 flow rate of 15 µL/min through the Lock Spray<sup>TM</sup> probe. We tentatively identified phenolic  
193 compounds on the basis of their molecular formula (as determined from exact mass  
194 measurements), fragment ions, and comparison with available databases such as the  
195 Dictionary of Natural Products (Chapman and Hall, CRC Informa, London; version 20.2)  
196 or ReSpect for Phytochemicals (Sawada et al., 2012). In total, 24 different phenolics were  
197 detected in cotton leaves, which were classified into different groups of phenolic



198 compounds, namely: flavonoids, condensed tannins, hydrolysable tannins, and lignins. We  
199 quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents,  
200 hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents. We  
201 achieved the quantification of these phenolic compounds by external calibration using  
202 calibration curves at 0.2, 0.8, 2, 5 and 20  $\mu\text{g}/\text{mL}$ . We calculated total phenolics as the sum  
203 of flavonoids, lignins, condensed tannins and hydrolysable tannins, and expressed  
204 concentrations of each phenolic group in  $\mu\text{g g}^{-1}$  tissue on a dry weight basis. Samples from  
205 one population were lost and therefore not included in the statistical analysis.

206

207 ***Geographic and climatic variables*** — Latitude and longitude of each cotton population  
208 were assessed using a Global Positioning System device (Garmin, Kansas, USA). To  
209 characterize the climatic conditions present at each site, we used a subset of the BioClim  
210 climate variables based on the geographical coordinates of each population (available at:  
211 <http://www.worldclim.org/>, version 2), namely: BIO1 (annual mean temperature,  $^{\circ}\text{C}$ ), BIO4  
212 (temperature seasonality, expressed as the standard deviation of temperature among  
213 months\*100), BIO5 (maximum temperature of the warmest month,  $^{\circ}\text{C}$ ), BIO6 (minimum  
214 temperature of the coldest month,  $^{\circ}\text{C}$ ), BIO12 (annual precipitation, mm), BIO13  
215 (precipitation of the wettest month, mm), BIO14 (precipitation of the driest month, mm),  
216 BIO15 (precipitation seasonality, expressed as standard deviation of precipitation across  
217 months) (Abdala-Roberts et al., 2016b; Moreira et al., 2018b). The procedures used to  
218 calculate these variables are based on interpolation methods which are fully described in  
219 Hijmans et al. (2005). WorldClim ver. 2 is based on data from 1970-2000, allowing us to  
220 test whether the recent climatic history of the sites correlated with current herbivory. Such

221 analyses imply a mismatch in the temporal scale of analysis for climate vs. herbivory data  
222 which is important to consider, since current leaf damage patterns may be influenced by  
223 shorter-term variation in weather conditions (e.g. during the current or previous season),  
224 which may deviate from longer-term climate trends. At the same time, analyses based on  
225 short-term climatic data may produce patterns that are not representative of longer-term  
226 climatic variation across sites, which is problematic since longer-term climatic variation  
227 will influence broad-scale spatial variation in herbivory and arthropod communities,  
228 independently of short-term fluctuations. In sum, broad-scale spatial variation in arthropod  
229 abundance is expected to result from a combination of long- and short-term climatic  
230 variation and we presume effects of site variation in climatic history on herbivory acting  
231 over and above effects of short-term climatic variation.

232

233 *Statistical analyses* — Climatic variables were previously summarized using Principal  
234 Component Analyses (PCA) to simplify the evaluation of climatic effects and avoid  
235 inflating Type I error due to multiple tests with individual climatic predictors (Abdala-  
236 Roberts et al., 2016b; Moreira et al., 2018b). The first principal component from PCA of  
237 temperature variables explained 68% of the variance in the four temperature variables  
238 across populations (“PC temperature” hereafter), and was positively related to mean annual  
239 temperature and maximum temperature of the warmest month. The first principal  
240 component from the PCA of precipitation variables explained 66% of the variance in the  
241 four precipitation variables across populations (“PC precipitation” hereafter), and was  
242 positively related to mean annual precipitation and precipitation of the wettest month. We  
243 used the standardized  $z$ -scores of the first PC in each case in the statistical analyses  
244 described below (multiple regressions and structural equation models).

245 First, we assessed population variation in leaf traits and herbivory with general  
246 linear models using data at the plant level. These models tested for an effect of population  
247 (fixed) on leaf pubescence, gossypol gland density, the concentration of phenolic  
248 compounds, and percent leaf area consumed by insect leaf-chewers. Second, we conducted  
249 an analysis based on piecewise structural equation modelling (SEM) based on population-  
250 level data (i.e. population means). We initially included all hypothesized associations  
251 between climatic factors, leaf defensive traits, and herbivory (following causal model, Fig.  
252 1), and then subsequently broke down this general model to test for specific relationships  
253 involving underlying correlates of plant traits and herbivory. To be consistent with the  
254 causal model, we also included the effect of latitude on climatic variables to illustrate the  
255 direction of latitudinal variation in abiotic conditions (longitude was excluded as  
256 preliminary analyses indicated that this variable was not correlated to either cotton traits or  
257 herbivory). Direct and indirect effects of latitude on plant traits and herbivory were not  
258 included to simplify the model (and reduce parameter load) and, more importantly, because  
259 this predictor is a proxy of climatic variables, and it is the latter which act upon and explain  
260 latitudinal gradients in plant defenses and herbivory. Conventional SEM estimates the  
261 effect of predictors on response variables simultaneously, while in piece-wise SEM the  
262 causal network is broken down into different independent linear regression models and then  
263 combined (Lefcheck, 2016). This approach allows to easily incorporate specific  
264 assumptions in each of the regression models that were included in the SEM (Lefcheck,  
265 2016). Direct effects in the SEM were estimated as standardized partial regression  
266 coefficients, whereas indirect effects were obtained by combining the specified coefficients  
267 for direct effects on both the predictor and the response. The significance of direct and  
268 indirect coefficients was assessed with t-tests. The goodness of fit of the general model was

269 evaluated with a ‘test of direct separation’ based on the Fisher’s C-test. Relationships  
270 between response and significant predictors variables were tested for correlation using the  
271 partial residuals as extracted from the piece-wise general model (Lefcheck, 2016). Gland  
272 density, leaf pubescence, and total phenolics were not significantly correlated (Pearson’s  $r$   
273  $< 0.27$ ,  $P > 0.19$ ), indicating that collinearity did not affect results from this analysis.  
274 Although the climatic PCs were significantly correlated ( $r = 0.78$ ,  $P < 0.0001$ ), this did not  
275 prevent detecting significant effects by these predictors (see *Results*), suggesting  
276 collinearity did not strongly influence the analysis.

277         Throughout the results section, we present means and SE as descriptive statistics.  
278 Leaf pubescence data at the plant-level (used to test for population variation in response  
279 variables) were log-transformed to achieve normality of residuals. Data were normally  
280 distributed without transformation in all the other cases. General linear mixed models and  
281 the PCA were conducted with PROC GLIMMIX and PROC FACTOR (rotation =  
282 varimax), respectively in SAS ver. 9.4 (SAS Institute, Cary, NC). The piecewise structural  
283 equation model was performed in R ver. 3.6.0 (R Core Team, 2018) using the  
284 *piecewiseSEM* package (Lefcheck, 2016). We used the *psem* function to obtain SEM fit  
285 parameters and the *partialResid* function to extract the partial effects of any relevant  
286 predictors on plant traits or herbivory accounting for all other covariates locally in each  
287 piece-wise model (Lefcheck, 2016).

288

## 289 **RESULTS**

290 We found significant variation among wild *G. hirsutum* populations in herbivory and in all  
291 the leaf defensive traits measured. Gossypol gland density varied up to two-fold (302.50 to  
292 616.09 glands  $\text{cm}^{-2}$ ), leaf pubescence ranged from 0 to over 1600 needles per  $\text{cm}^2$ , and

293 population variation in total phenolics varied up to 5.2-fold (702.34 to 3694.81  $\mu\text{g g}^{-1}$ )  
294 (Table 1; Appendix B *supplementary material*). In addition, leaf herbivory varied up to 5.8-  
295 fold among populations (9.22 to 52.13% leaf area consumed) (Table 1; Appendix B).

296 Results from the piece-wise SEM analysis showed expectedly significant negative  
297 effects of latitude on both temperature and precipitation (Fig. 3). Importantly, there were  
298 significant effects of climatic factors on cotton defenses and herbivory (Fig. 3), whereby  
299 precipitation negatively affected leaf pubescence (Fig. 3, 4A) and positively affected  
300 herbivory (Fig. 3, 4B), and temperature negatively affected herbivory (Fig. 3, 4C). There  
301 was also evidence that plant defenses were associated with herbivory, as leaf pubescence  
302 (but not phenolics or gossypol glands) exhibited a significant negative association with leaf  
303 damage (Fig. 3, 4D). Finally, there were no significant indirect effects of either temperature  
304 (coefficient = -0.46, P = 0.13) or precipitation (coefficient = 0.21, P = 0.28) (via cotton  
305 traits) on herbivory.

306

## 307 **DISCUSSION**

308 There was substantial population variation in wild cotton leaf defensive traits and herbivore  
309 damage along this species' coastal distribution in the Yucatan Peninsula. Our findings  
310 indicated that leaf pubescence, but not gossypol gland density or phenolics, was  
311 significantly negatively associated with population variation in insect leaf herbivory. We  
312 also found strong direct effects of temperature and precipitation on leaf herbivory;  
313 herbivore pressure on wild cotton was lower in warmer and drier sites (i.e. more arid  
314 habitats). In addition, precipitation was negatively associated with leaf pubescence. Finally,  
315 despite significant effects of climate on leaf pubescence and significant effects of this trait

316 on herbivory, we found no evidence of indirect effects (via pubescence) of climate on leaf  
317 damage. Overall, these findings demonstrate concurrent bottom-up effects of leaf  
318 pubescence and climatic factors (precipitation and temperature) on insect leaf herbivory  
319 across populations of wild *G. hirsutum*.

320

### 321 ***Cotton traits correlated with spatial variation in herbivory***

322 Our results showed no significant associations between gossypol gland density and  
323 phenolic compounds with insect leaf herbivory across populations of wild cotton. These  
324 results run counter to previous work showing that gland density and terpenoid  
325 concentrations (e.g. McAuslane and Alborn, 1997; Stipanovic et al., 2006; Opitz et al.,  
326 2008), as well as phenolic compounds (e.g. Mansour et al., 1997; Nix et al., 2017) play a  
327 role in resistance against insect herbivory in this plant species. Not all studies, however,  
328 support a role in direct resistance of these chemical traits (e.g. McAuslane and Alborn,  
329 1998; Agrawal and Karban, 2000), suggesting that their defensive function is contingent on  
330 the herbivore species or guild under study or on abiotic conditions. Our measurements of  
331 leaf herbivory included damage by different species of chewing insects (mainly  
332 grasshoppers, beetles, and caterpillars), and thus may have lacked the appropriate resolution  
333 to address whether pigment glands are associated with damage by specific herbivores (e.g.  
334 dietary specialist species). In addition, previous work has found that gland density and  
335 terpenoid production (per gland) both contribute to resistance against insect herbivores and  
336 should therefore be considered separated and individually measured (Opitz et al., 2008).  
337 Future work must consider true terpenoid concentrations in order to fully test for effects of  
338 these compounds on herbivory.

339 Rather than gland density or phenolics, results pointed at leaf pubescence (i.e.

340 trichome needle density) as the main leaf trait influencing herbivory across populations of  
341 *G. hirsutum*. The defensive role of trichomes has long been studied in plants (Levin, 1973),  
342 and work with both wild (e.g. Agrawal, 1999; Valverde et al., 2001; Abdala-Roberts et al.,  
343 2016b) and cultivated (e.g. Wilkens et al., 1996; Kaplan et al., 2009) species has reported  
344 on their role in deterring herbivores and reducing insect damage. In *G. hirsutum*, the role of  
345 trichomes in direct defense has received little attention and most work has involved  
346 cultivated varieties (Mahtews, 1989). The fact that trichomes were associated with our  
347 broad-spectrum measurements of leaf damage suggests a defensive role against a relatively  
348 diverse fauna of generalist insect herbivores feeding on wild *G. hirsutum*. The primacy of  
349 trichomes as a driver of herbivory in wild cotton should, however, be taken with caution as  
350 our analyses are of a correlational nature, and because population variation in the  
351 concentration of gossypol and other related terpenoids which are known to provide  
352 herbivore resistance in this species (at least for cultivated varieties) remains to be assessed.

353

#### 354 *Effects of climate on spatial variation in leaf traits and herbivory*

355 We found evidence for climatic bottom-up control over population variation in leaf  
356 pubescence *G. hirsutum*. This trait increased with aridity, suggesting that increasing water  
357 stress and reduced resource availability increases investment in this putative defensive trait  
358 (following Resource Availability Hypothesis; Coley et al., 1985). Alternatively, this  
359 pubescence may play a role in plant tolerance to abiotic stress, with previous work with  
360 other plant species reporting that leaf trichomes play a function in abiotic tolerance by  
361 influencing plant evapotranspiration and water use efficiency (e.g. Hare and Elle, 2001),  
362 and this could also explain. Likewise, although not significant in the piecewise SEM  
363 (coefficient =  $0.53 \pm 0.29$   $P = 0.09$ ), gland density also tended to increase with temperature.

364 Terpenes have been also shown to play a role in mediating plant responses to abiotic stress  
365 and recent work has found increasing leaf terpene concentrations under warmer and drier  
366 climatic conditions both within (Jamieson et al., 2012) and across plant populations (Pratt  
367 et al., 2014). Further work addressing the role of gossypol glands and trichomes in  
368 tolerance to abiotic stress in wild *G. hirsutum* is needed. It should also be noted that latitude  
369 and longitude were significant predictors of pubescence after accounting for climatic  
370 factors, suggesting that other unmeasured factors varying across these geographic axes  
371 influenced this trait.

372         There was a strong direct influence of climatic conditions on cotton population  
373 variation in insect herbivory. Leaf damage decreased with increasing aridity, which agrees  
374 with previous studies reporting stronger herbivore pressure with increasing precipitation  
375 (e.g. along latitudinal gradients; Schemske et al., 2009). Studies conducted at broader  
376 spatial scales, including a greater range of variation in temperature (including temperate  
377 latitudes), have found inverse patterns. For example, Zhang et al. (2016) recently reported a  
378 positive association between herbivory and temperature in the Northern Hemisphere,  
379 whereas the inverse pattern held for the Southern Hemisphere. In combination, these results  
380 highlight that the magnitude and sign of climate-herbivory associations can vary across  
381 regions depending on the range, upper and lower limits of climatic conditions considered,  
382 as well as biotic factors such as variation in plant-herbivore species composition and life  
383 history traits (Abdala-Roberts et al., 2016b; Zhang et al., 2016). In our case, a strong  
384 correlation with temperature was found despite relatively low variation across sites (range  
385 for mean annual temperature: 1.2°C), and our findings agree with previous work also  
386 showing negative effects of temperature on spatial variation in insect seed predation for a  
387 perennial herb in the same region (Moreira et al., 2015; Abdala-Roberts et al., 2016b). The



388 Yucatan Peninsula has a subtropical climate and cotton populations found along the  
389 northern coast are exposed to more extreme temperatures during the dry and wet season  
390 (>35°C maximum monthly averages) relative to populations on the southwest coast, which  
391 likely results in greater thermal stress on insects and reduced herbivory rates.

392 Overall we found no evidence for indirect effects of temperature or precipitation on  
393 either insect leaf herbivory. This null result took place even though temperature  
394 significantly affected leaf pubescence and this plant defensive trait significantly affected  
395 leaf herbivory. These findings run counter to recent work emphasizing the importance of  
396 bottom-up plant-mediated effects of abiotic factors on consumers (Rosenblatt and Schmitz,  
397 2016), and previous work reporting significant indirect effects of climate on insect  
398 herbivore abundance, diversity and damage (Pratt et al., 2017; Moreira et al., 2018b). It is  
399 important to note, however, that indirect effects are frequently hard to statistically detect  
400 and this may have therefore required a larger population sample size. This may have been  
401 especially the case given limited statistical power in our SEM. Thus, we caution on ruling  
402 out plant-mediated indirect effects of climate on herbivory on wild cotton.

403

#### 404 ***Concluding remarks and further work***

405 Overall, our results indicate a key role of leaf defensive traits and climatic conditions in  
406 shaping geographic patterns of insect leaf herbivory on wild *G. hirsutum*. Leaf pubescence  
407 and climatic factors appeared to exert particularly strong effects on insect leaf herbivory,  
408 and in the latter case effects of climate were primarily direct (rather than indirect via leaf  
409 traits such as pubescence), though low power may have prevented the detection of such  
410 effects. Moreover, further work must consider surveys of predator abundance, particularly  
411 ants and spiders which are the two dominant groups of predators on wild cotton plants, as

412 well as traits (e.g. volatile compounds) potentially mediating recruitment of these natural  
413 enemies. Assessing top-down control and its associated traits in wild cotton under natural  
414 conditions is necessary to achieve a complete multi-trophic assessment of biotic controls  
415 over geographic herbivory (e.g. Björkman et al., 2010). Overall, our results imply that a  
416 robust understanding of bottom-up controls over geographic variation in herbivory requires  
417 addressing the simultaneous and independent effects of plant defensive traits and climatic  
418 factors. Combining observational approaches such as the present study with experimental  
419 manipulations of herbivory and abiotic factors will yield the best insight into the  
420 mechanisms governing plant-herbivore interactions.

421

#### 422 **ACKNOWLEDGEMENTS**

423 We thank K. Baas, B. Pérez, N. Salinas, R. Silveira and M. S. Campañó for assistance in  
424 the field and laboratory. We thank J. Lau and two anonymous reviewers for comments on a  
425 previous version of the manuscript. This research was financially supported by a Swiss seed  
426 money grant (for collaborations with Latin America) awarded to TCJT and VPT, and by a  
427 Spanish National Research Grant (AGL2015-70748-R) to XM.

428

#### 429 **LITERATURE CITED**

430 ABDALA-ROBERTS, L. AND K. A. MOONEY. 2015. Plant and herbivore evolution within the  
431 trophic sandwich. *In*: Hanley, T. and K. La Pierre [eds.], *Trophic Interactions:  
432 Bottom-up and Top-down Interactions in Aquatic and Terrestrial Ecosystems*, 340-  
433 364. Cambridge University Press, Cambridge.

434 ABDALA-ROBERTS, L., S. RASMANN, J. C. BERNY-MIER Y TERÁN, F. COVELO, G. GLAUSER,  
435 AND X. MOREIRA. 2016a. Biotic and abiotic factors associated with altitudinal

436 variation in plant traits and herbivory in a dominant oak species. *American Journal of*  
437 *Botany* 103:2070-2078.

438 ABDALA-ROBERTS, L., X. MOREIRA, S. RASMANN, V. PARRA-TABLA, AND K. A. MOONEY.  
439 2016b. Test of biotic and abiotic correlates of latitudinal variation in plant defenses  
440 in the perennial herb *Ruellia nudiflora*. *Journal of Ecology* 104:580-590.

441 ABDALA-ROBERTS, L., B. PÉREZ-NIÑO, X. MOREIRA, V. PARRA-TABLA, L. GRANDI, G.  
442 GLAUSER, B. BENREY, AND T. C. J. TURLINGS. 2019. Effects of early-season insect  
443 herbivory on subsequent pathogen infection and ant abundance on wild cotton  
444 (*Gossypium hirsutum*). *Journal of Ecology* 107: 1518-1529.

445 AGRAWAL, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several  
446 herbivores and plant fitness. *Ecology* 80:1713-1723.

447 AGRAWAL, A. A. 2011 Current trends in the evolutionary ecology of plant defense.  
448 *Functional Ecology* 25:420-432.

449 AGRAWAL, A. AND R. KARBAN. 2000. Specificity of constitutive and induced resistance:  
450 pigment glands influence mites and caterpillars on cotton plants. *Entomologia*  
451 *Experimentalis et Applicata* 96:39-49.

452 ANSTETT, D. N., K. A. NUNES, C. BASKETT, AND P. M. KOTANEN. 2016. Sources of  
453 controversy surrounding latitudinal patterns in herbivory and defense. *Trends in*  
454 *Ecology and Evolution* 31:789-802.

455 BJÖRKMAN, C., Å. BERGGREN, AND H. BYLUND. 2011. Causes behind insect folivory  
456 patterns in latitudinal gradients. *Journal of Ecology* 99:367-369.

457 COLEY, P. D., J. P. BRYANT, AND F. S. CHAPIN. 1985. Resource availability and plant  
458 antiherbivore defense. *Science* 230:895-899.

459 D'ECKENBRUGGE G. C. AND J. M. LACAPE. 2014. Distribution and differentiation of wild,

460 feral, and cultivated populations of perennial upland cotton (*Gossypium hirsutum*  
461 L.) in Mesoamerica and the Caribbean. *PLoS ONE* 9:e107458.

462 ENDARA, M. J. AND P. D. COLEY. 2011. The resource availability hypothesis revisited: a  
463 meta-analysis. *Functional Ecology* 25:389-398.

464 GRIPENBERG, S. AND T. ROSLIN. 2007. Up or down in space? Uniting the bottom-up versus  
465 top-down paradigm and spatial ecology. *Oikos* 116:181-188.

466 HAGENBUCHER, S., D. M. OLSON, J. R. RUBERSON, F. L. WÄCKERS, AND J. ROMEIS, J. 2013.  
467 Resistance mechanisms against arthropod herbivores in cotton and their interactions  
468 with natural enemies. *Critical Reviews in Plant Sciences* 32:458-482.

469 HAHN, P. G. AND J. L. MARON. 2016. A framework for predicting intraspecific variation in  
470 plant defense. *Trends in Ecology and Evolution* 31:646-656.

471 HARE, J. D. AND E. ELLE. 2001. Geographic variation in the frequencies of trichome  
472 phenotypes of *Datura wrightii* and correlations with annual water deficit. *Madroño*  
473 48:33-37.

474 HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high  
475 resolution interpolated climate surfaces for global land areas. *International Journal*  
476 *of Climatology* 25:1965-1978.

477 HUNTER, M. D. AND P. W. PRICE. 1992. Playing chutes and ladders: heterogeneity and the  
478 relative roles of bottom-up and top-down forces in natural communities. *Ecology*  
479 73: 724-732.

480 JAMIESON, M., A. M. TROWBRIDGE, K. F. RAFFA, AND R. L. LINDROTH. 2012. Consequences  
481 of climate warming and altered precipitation patterns for plant-insect and  
482 multitrophic interactions. *Plant Physiology* 160:1719-1727.

483 JOHNSON, M. T. J. AND S. RASMANN. 2011. The latitudinal herbivory-defence hypothesis

484 takes a detour on the map. *New Phytologist* 191:589-592.

485 KAPLAN, I., G. P. DIVELY, AND R. F. DENNO. 2009. The costs of anti-herbivore defense  
486 traits in agricultural crop plants: a case study involving leafhoppers and trichomes.  
487 *Ecological Applications* 19:864-872.

488 LEFCHECK, J. S. 2016. piecewiseSEM: Piecewise structural equation modelling in r for  
489 ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7:573-579.

490 LEVIN, D. A. 1973. The role of trichomes in plant defence. *Quarterly Review of Biology*  
491 48:3-15.

492 MANSOUR, M. H., N. M. ZOHDY, S. E. ELGENGAIHI, AND A. E. AMR. 1997. The relationship  
493 between tannins concentration in some cotton varieties and susceptibility to piercing  
494 sucking insects. *Journal of Applied Entomology* 121:321-325.

495 MATTHEWS, G. A. 1989. Cotton insect pests and their management. Longman Scientific and  
496 Technical and John Wiley & Sons, New York.

497 MCAUSLANE, H. J. AND H. T. ALBORN. 1997. Systemic induction of terpenoid aldehydes in  
498 cotton pigment glands by feeding of larval *Spodoptera exigua*. *Journal of Chemical*  
499 *Ecology* 23:2861-2879.

500 MCAUSLANE, H. J. AND H. T. ALBORN. 1998. Systemic induction of allelochemicals in  
501 glanded and glandless isogenic cotton by *Spodoptera exigua* feeding. *Journal of*  
502 *Chemical Ecology* 24:399-416.

503 MCCALL, P. J., T. C. TURLINGS, J. LOUGHRIN, A. T. PROVEAUX, AND J. H. TUMLINSON.  
504 1994. Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.)  
505 seedlings. *Journal of Chemical Ecology* 20:3039-3050.

506 MOLES, A.T., S.O. BONSER, A.G.B. POORE, I.R. WALLIS, AND W.J. FOLEY. 2011. Assessing  
507 the evidence for latitudinal gradients in plant defence and herbivory. *Functional*

508           *Ecology* 25:380-388.

509 MOREIRA, X., L. ABDALA-ROBERTS, V. PARRA-TABLA, AND K. A. MOONEY. 2015.

510           Latitudinal variation in herbivory: Influences of climatic drivers, herbivore identity,

511           and natural enemies. *Oikos* 124:1444-1452.

512 MOREIRA, X., W. PETRY, K. A. MOONEY, S. RASSMAN, AND L. ABDALA-ROBERTS. 2018a.

513           Elevational gradients in plant defense and herbivory: recent advances in the field

514           and prospects for future research. *Ecography* 41:1485-1496.

515 MOREIRA, X., B. CASTAGNEYROL, L. ABDALA-ROBERTS, J. C. BERNY-MIER Y TERÁN, B. G.

516           TIMMERMANS, H. H. BRUUN, F. COVELO, G. GLAUSER, S. RASMANN, AND A. TACK.

517           2018b. Latitudinal variation in plant chemical defences drives latitudinal patterns of

518           leaf herbivory. *Ecography* 41:1124-1134.

519 MOREIRA, X., K. A. MOONEY, S. RASMANN, W. K. PETRY, A. CARRILLO-GAVILÁN, R. ZAS,

520           AND L. SAMPEDRO. 2014. Trade-offs between constitutive and induced defences

521           drive geographical and climatic clines in pine chemical defences. *Ecology Letters*

522           17:537-546.

523 NIX, A., C. PAULL, AND M. COLGRAVE. 2017. Flavonoid profile of the cotton plant,

524           *Gossypium hirsutum*: A Review. *Plants* 6:43.

525 OOSTERHUIS D. M. AND J. JERNSTEDT. 1999. Morphology and anatomy of the cotton plant..

526           In: Smith, C. W. and J. T. Cothren [eds.], Cotton: Origin, History, Technology and

527           Production, 175-206. John Wiley and Sons, New York.

528 OPITZ, S., G. KUNERT, AND J. GERSHENZON, J. 2008. Increased terpenoid accumulation in

529           cotton (*Gossypium hirsutum*) foliage is a general wound response. *Journal of*

530           *Chemical Ecology* 34:508-522.

531 PEARSE, I. S. AND A. L. HIPPI. 2012. Global patterns of leaf defenses in oak species.

532           *Evolution* 66:2272-2286.

533 PRATT, J. D., K. KEEFOVER-RING, L. Y. LIU, AND K. A. MOONEY. 2014. Genetically based  
534           latitudinal variation in *Artemisia californica* secondary chemistry. *Oikos* 123:953-  
535           963.

536 PRATT, J. D., A. DATU, T. TRAN, D. C. SHENG, AND K. A. MOONEY. 2017. Genetically based  
537           latitudinal clines in *Artemisia californica* drive parallel clines in arthropod  
538           communities. *Ecology* 98:79-91.

539 R CORE TEAM. 2018. R: A language and environment for statistical computing. R  
540           Foundation for Statistical Computing, Vienna, Austria. URL [http://www.R-](http://www.R-project.org/)  
541           project.org/.

542 RHOADES, D. F. 1979. Evolution of plant chemical defense against herbivores. *In*:  
543           Rosenthal, G. A. and D. H. Janzen [eds.], *Herbivores: their Interaction With*  
544           *Secondary Plant Metabolites*, 4–54. Academic Press, New York.

545 ROSENBLATT, A. E. AND O. J. SCHMITZ. 2016. Climate change, nutrition, and bottom-up and  
546           top-down food web processes. *Trends in Ecology and Evolution* 31:965-975.

547 RUDGERS, J. A. S. Y., STRAUSS, AND J. F. WENDEL. 2004. Trade-offs among anti-herbivore  
548           resistance traits: insights from *Gossypieae* (Malvaceae). *American Journal of*  
549           *Botany* 91:871-880.

550 SAS. 2014. SAS, version 9.4. SAS Institute Inc., North Carolina. USA.

551 SAWADA, Y., R. NAKABAYASHI, Y. YAMADA, M. SUZUKI, M. SATO, A. SAKATA, K.  
552           AKIYAMA, T. SAKURAI, F. MATSUDA, T. AOKI, M. Y. HIRAI, AND K. SAITO. 2012.  
553           RIKEN tandem mass spectral database (ReSpect) for phytochemicals: A plant-  
554           specific MS/MS-based data resource and database. *Phytochemistry* 82:38-45.

555 SCHEMSKE, D. W., G. G. MITTELBACH, H. V. CORNELL, J. M. SOBEL, AND K. ROY. 2009. Is

556           there a latitudinal gradient in the importance of biotic interactions? *Annual Review of*  
557           *Ecology and Systematics* 40:245-269.

558 STIPANOVIC, R. D., J. D. LOPEZ, M. K. DOWD, L. S. PUCKHABER, AND S. E. DUKE. 2006.  
559           Effect of racemic and (+)- and (-)-gossypol on the survival and development of  
560           *Helicoverpa zea* larvae. *Journal of Chemical Ecology* 32:959-968.

561 VALVERDE, P. L., J. FORNONI, AND J. NÚÑEZ-FARFÁN. 2001. Defensive role of leaf  
562           trichomes in resistance to herbivorous insects in *Datura stramonium*. *Journal of*  
563           *Evolutionary Biology* 14:424-432.

564 WÄCKERS, F. L. AND T. M. BEZEMER. 2003. Root herbivory induces an above-ground  
565           indirect defence. *Ecology Letters* 6:9-12.

566 WENDEL, J. F., C. L. BRUBAKER, AND A. E. PERCIVAL. 1992. Genetic diversity in  
567           *Gossypium hirsutum* and the origin of upland cotton. *American Journal of Botany*  
568           79:1291-1310.

569 ZHANG, S., Y. ZHANG, AND K. MA. 2016. Latitudinal variation in herbivory: Hemispheric  
570           asymmetries and the role of climatic drivers. *Journal of Ecology* 104:1089-1095.

571  
572  
573  
574  
575  
576  
577  
578  
579



580 **Table 1.** Descriptive statistics (population mean range and SE) for *G. hirsutum* population  
 581 variation in leaf traits, namely: gossypol gland density (number of glands cm<sup>-2</sup>), pubescence  
 582 (number of trichome needles cm<sup>-2</sup>), total phenolics (μg g<sup>-1</sup> d.w.), and insect leaf herbivory  
 583 (% leaf area consumed). Data are from 26 populations sampled in northern Yucatan and  
 584 Campeche (SE Mexico); we excluded data for phenolics for one population (see text). *F*-  
 585 and *P*-values are from general linear models testing for an effect of population. Significant  
 586 (*P* < 0.05) results are typed in bold.

587

| 588 | Variable        | Mean range                         | <i>F</i> | <i>P</i>       |
|-----|-----------------|------------------------------------|----------|----------------|
| 589 | Gland density   | 302.50 ± 19.33 – 616.09 ± 63.71    | 4.58     | < <b>0.001</b> |
|     | Leaf pubescence | 0 – 1681.88 ± 116.24               | 19.69    | < <b>0.001</b> |
| 590 | Total phenolics | 702.34 ± 101.06 – 3694.81 ± 145.14 | 9.75     | < <b>0.001</b> |
| 591 | Herbivory       | 9.22 ± 0.87 – 52.13 ± 8.06         | 9.38     | < <b>0.001</b> |

592

593

594

595

596

597

598

599

600

601

602

603 **FIGURE LEGENDS**

604 **Figure 1.** Causal diagram showing predicted direct and indirect associations among  
605 latitude, climate, plant defenses, and herbivory. Arrow A: Mean temperature and mean  
606 annual precipitation increase towards lower latitudes, depicting a negative association;  
607 arrow B: direct negative effect of plant defensive traits on herbivory (i.e. resistance); arrow  
608 C: direct effect of climate on herbivory, with precipitation increasing herbivory and  
609 temperature decreasing or increasing herbivory depending on the range and extremes of  
610 temperature variation; arrow D: direct effects of climate on plant defences, with  
611 precipitation increasing plant growth which then lowers defences (via allocation  
612 constraints), and temperature decreasing growth and thus increasing defences (negative or  
613 positive association, depending on climatic variable; other mechanisms may lead to  
614 different outcomes). **Not shown, but also possible, is the indirect effect of climate on  
615 herbivory via effect on plant defences.**

616

617 **Figure 2.** Map indicating the location of the wild cotton (*Gossypium hirsutum*) populations  
618 (N = 26) sampled along the coast of the Yucatan Peninsula (México).

619

620 **Figure 3.** Diagram showing results from an analysis based on piecewise structural equation  
621 modelling testing for associations among latitude, climatic factors (temperature- and  
622 precipitation-related variables), leaf putative defensive traits (total phenolics, pubescence,  
623 gossypol glands), and insect leaf herbivory (percent leaf area consumed) on wild cotton  
624 (*Gossypium hirsutum*). Values used in this analysis were population means (N = 26).  
625 Climatic variables represent z-score values from a principal components analysis  
626 summarizing a suite of variables associated to precipitation or temperature. Values next to

627 each arrow are path coefficients (i.e. standardized regression coefficients). Continuous  
628 arrows indicate positive associations whereas broken arrows indicate negative associations;  
629 we also tested for and did not find significant indirect effects of either temperature or  
630 precipitation on herbivory (arrows not shown for ease of visualization). The model also  
631 accounted for co-variation between temperature and precipitation as well as between leaf  
632 defensive traits, but these estimates are not shown for ease of visualization. Significant ( $*P$   
633  $< 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ) and non-significant path coefficients (text and arrows) are  
634 in black and grey, respectively. Explained variance: pubescence=0.16; phenolics=0.46;  
635 gossypol glands=0.19; herbivory=0.52. Fisher's  $C = 5.93$ ,  $P = 0.431$ ,  $AICc = 43.93$ .

636

637 **Figure 4.** Partial residual plots from the piece-wise structural equation model showing  
638 relationships between leaf pubescence (number of trichome needles  $\text{cm}^{-2}$ ) and precipitation  
639 (A), and between insect leaf herbivory with precipitation (B), temperature (C), and leaf  
640 pubescence (D). Predicted relationships come from simple linear regressions and the slope  
641 of each relationship is the partial correlation coefficient ( $r^2$ ) for the effect of the predictor  
642 on the response. Precipitation and temperature values used are z-scores from the first axis  
643 of two principal components analyses summarizing a suite of relevant precipitation- and  
644 temperature-related variables (*see statistical analyses*).

Figure 1.

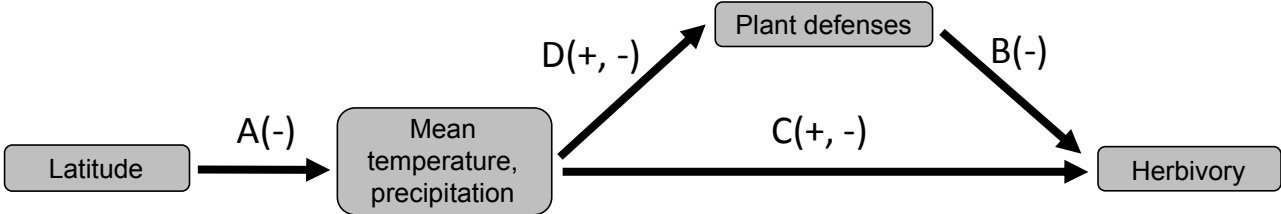


Figure 2.

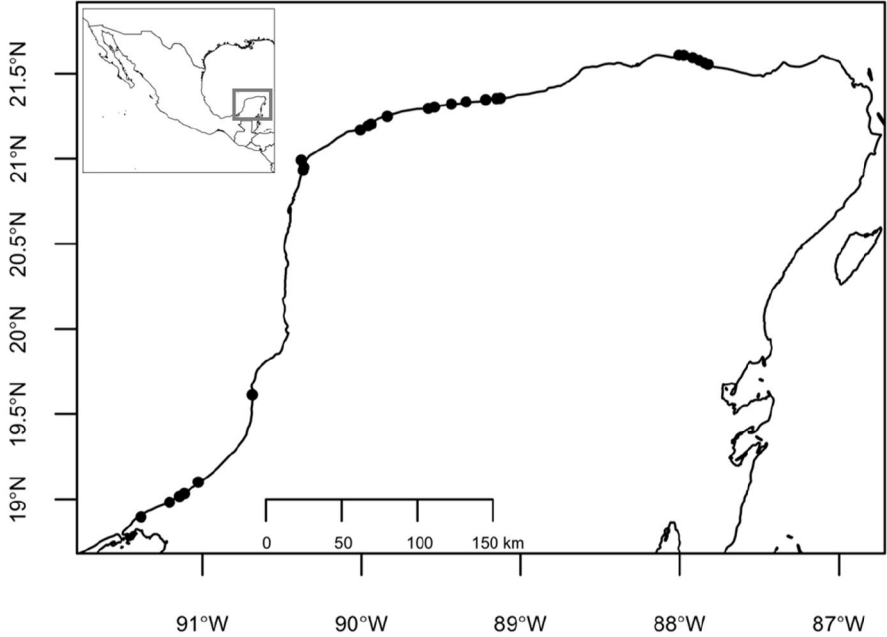


Figure 3.

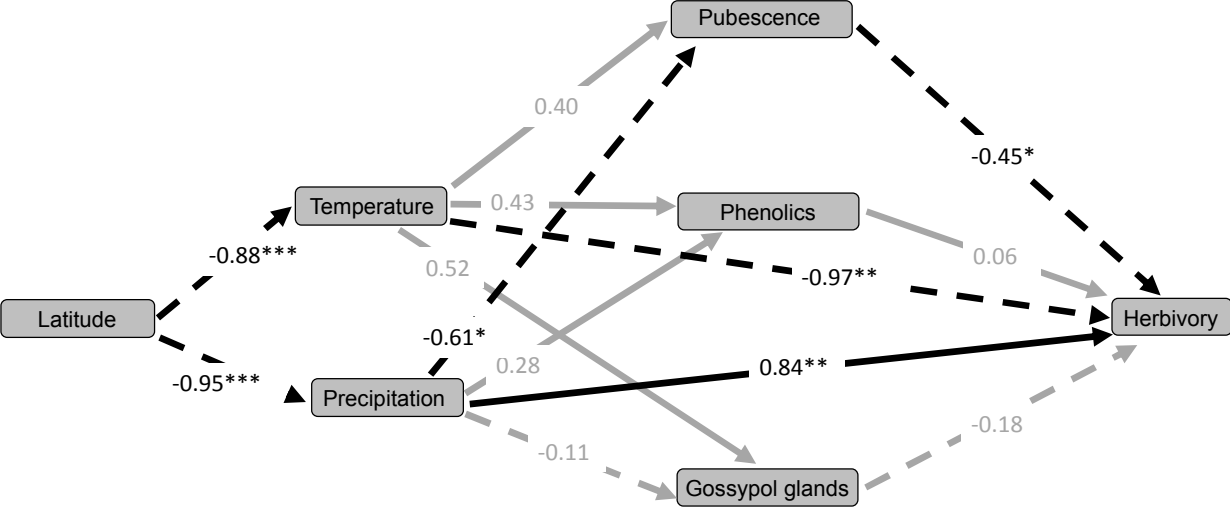


Figure 4.

