Bottom-up control of geographic variation in insect herbivory on wild cotton (*Gossypium hirsutum*) by plant defenses and climate

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PREMISE: The occurrence and amount of herbivory are shaped by bottom-up forces, primarily plant traits (e.g., defenses), and by abiotic factors. Addressing these concurrent effects in a spatial context has been useful in efforts to understand the mechanisms governing variation in plant–herbivore interactions. Still, few studies have evaluated the simultaneous influence of multiple sources of bottom-up variation on spatial variation in herbivory.

METHODS: We tested to what extent chemical (phenolics, production of terpenoid glands) and physical (pubescence) defensive plant traits and climatic factors are associated with variation in herbivory by leaf-chewing insects across populations of wild cotton (*Gossypium hirsutum*).

RESULTS: We found substantial population variation in cotton leaf defenses and insect leaf herbivory. Leaf pubescence, but not gossypol gland density or phenolic content, was significantly negatively associated with herbivory by leaf-chewing insects. In addition, there were direct effects of climate on defenses and herbivory, with leaf pubescence increasing toward drier conditions and leaf damage increasing toward wetter and cooler conditions. There was no evidence, however, of indirect effects (via plant defenses) of climate on herbivory.

CONCLUSIONS: These results suggest that spatial variation in insect herbivory on wild *G. hirsutum* is predominantly driven by concurrent and independent influences of population variation in leaf pubescence and climatic factors.

KEY WORDS abiotic factors; herbivory; leaf pubescence; Malvaceae; plant chemistry; spatial variation; wild cotton.

Research has long recognized that herbivory by insects is strongly determined from the "bottom-up" by factors such as plant defenses and abiotic variables (Rhoades, 1979; Agrawal, 2011). The study of these factors in a geographic context (e.g., along ecological gradients) has been particularly useful to understand the mechanisms of bottom-up control on plant–herbivore interactions (Hunter and Price, 1992; Gripenberg and Roslin, 2007; Abdala-Roberts and Mooney, 2015). Notably, studies along latitudinal or altitudinal gradients have commonly found higher levels of plant defenses and herbivory toward warmer and wetter climates present at lower latitudes and elevations (Fig. 1, arrow A; reviewed by Anstett et al., 2016; Moreira et al., 2018b). These patterns have been attributed to greater herbivore abundance and diversity under seasonally less variable, warmer and wetter climates, resulting in more intense plant–herbivore interactions and thus stronger selection on plant qualitative or quantitative defenses which in turn reduces herbivory (Fig. 1, arrow B; Schemske et al., 2009; Zhang et al., 2016; but see Moles et al., 2011).

Abiotic factors vary substantially across space, shaping concomitant inter- and intraspecific plant variation in defensive traits and herbivory (Johnson and Rasmann, 2011; Pearse and Hipp, 2012; Hahn and Maron, 2016; Moreira et al., 2018b). For instance, climatic conditions may directly affect herbivore population size or behavior and thus herbivory rates (Fig. 1, arrow C). In addition,
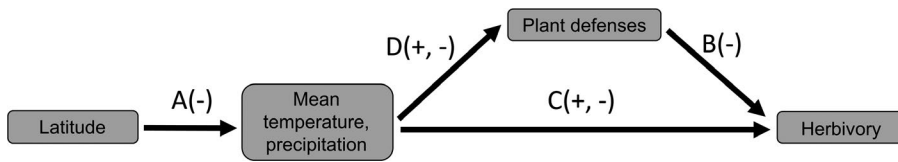
FIGURE 1. Causal diagram showing predicted direct and indirect associations among latitude, climate, plant defenses, and herbivory. Arrow A: Mean temperature and mean annual precipitation increase toward lower latitudes, depicting a negative association; arrow B: direct negative effect of plant defensive traits on herbivory (i.e., resistance); arrow C: direct effect of climate on herbivory, with precipitation increasing herbivory and temperature decreasing or increasing herbivory depending on the range and extremes of temperature variation; arrow D: direct effects of climate on plant defenses, with precipitation increasing plant growth which then lowers defenses (via allocation constraints), and temperature decreasing growth and thus increasing defenses (negative or positive association, depending on climatic variable; other mechanisms may lead to different outcomes). Not shown, but also possible, is the indirect effect of climate on herbivory via effect on plant defences.

climatic factors may affect plant defenses, e.g., via allocation constraints; increased precipitation (and thereby higher resource availability) favors greater plant growth, which then leads to lowered plant defenses due to underlying growth-defense trade-offs (Fig. 1, arrow D; resource availability hypothesis, Coley et al., 1985; Endara and Coley, 2011). By the same logic, increasing temperature can reach a point where plant growth is reduced, which in turn favors increased defenses; alternatively, warming could lower defenses due to thermal stress reducing defense allocation (Fig. 1, arrow D). These climate-mediated effects on defenses may in turn indirectly (positively or negatively, depending on the direction of the direct effects of climatic variables on plant defenses) shape geographic variation in herbivory (e.g., Pratt et al., 2017; Moreira et al., 2018b). Although a number of studies have assessed these relationships, few have simultaneously assessed the relative influences of variation in plant traits and abiotic factors on herbivory, thus limiting our understanding on the mechanisms underlying bottom-up control of spatial variation in herbivory.

Wild cotton, *Gossypium hirsutum* L. (Malvaceae), is a perennial plant that is naturally distributed along the northern and western coastal scrublands of the Yucatan Peninsula (D'Eeckenbrugge and Lacape, 2014). This species possesses physical (e.g., trichomes) and chemical (e.g., terpenoids, phenolics) defenses against herbivores (Abdala-Roberts et al., 2019) and is exposed to substantial variation in climatic conditions along its relatively narrow coastal distribution range (e.g. >2-fold variation in mean annual precipitation along 2.5° latitude). On the basis of these biotic and abiotic features, we sought to disentangle the associations between insect herbivory and plant defensive traits and climatic factors in this species. Our goals were to (1) assess geographic variation in leaf defensive traits and insect leaf herbivory across populations of wild cotton, (2) determine whether plant defensive traits are associated with (and presumably underlie) population variation in herbivory, and (3) evaluate the effect of variation in climatic factors on population variation in cotton defensive traits and herbivory and whether effects of climate on herbivory are mediated (indirectly) by abiotic controls on plant defensive traits. Although a numerous studies have tested for spatial variation (e.g., latitudinal, elevational) in herbivory or plant defenses, fewer have measured herbivory *and* defenses, and only a handful have combined data on herbivory, plant defenses, and abiotic factors. In analyzing all three, this study provides a robust assessment of the independent contribution of multiple bottom-up factors concurrently shaping geographic variation in plant–herbivore interactions.

MATERIALS AND METHODS

Study species

Gossypium hirsutum is a perennial shrub distributed throughout Central America, Mexico, and the Caribbean (Wendel et al., 1992; Oosterhuis and Jernstedt, 1999), and current data implicate southeastern Mexico as it center of origin (D'Eeckenbrugge and Lacape, 2014). Naturally occurring, wild populations of this species are found along the northern coast of the Yucatan Peninsula (Mexico), where it grows with other native species of the coastal scrubland or sand dune vegetation (D'Eeckenbrugge and Lacape, 2014). At these sites, a number of native insect herbivores attack wild cotton, and the most important feeding guild are leaf chewers among which caterpillars and beetles are the particularly common (T. Quijano-Medina, personal observation). Sap feeders (mainly bugs and leafhoppers) are relatively less common (L. Abdala-Roberts, personal observation).

Most research on direct defenses in *G. hirsutum* comes from studies with domesticated varieties (Hagenbucher et al., 2013), whereas fewer studies have been conducted on wild populations of this species (for work with other congeneric wild species, see Rudgers et al., 2004). Specifically, *G. hirsutum* plants produce pigment glands throughout the surface of leaves, stems, and fruits, and these contain gossypol and other related compounds (mainly terpenoid aldehydes), which act as deterrents to insect herbivore feeding (McAuslane et al., 1997; Rudgers et al., 2004; Stipanovic et al., 2006). Likewise, phenolic compounds have also been reported to confer direct resistance against insect herbivores in this species (Mansour et al., 1997; Nix et al., 2017; Abdala-Roberts et al., 2019). In addition, wild cotton produces physical defenses such as nonglandular trichomes putatively related to herbivore resistance in this species (for work with other cotton species, see Rudgers et al., 2004). On the other hand, *G. hirsutum* also produces extrafloral nectar and volatile compounds, which recruit a number of species of ants and parasitoids and possibly play a role in indirect defence (McCall et al., 1994; Wäckers and Bezemer, 2003; Rudgers et al., 2004). To date, however, the role of these volatiles and extrafloral nectar in indirect defence has not been yet evaluated in wild populations.

Population sampling

In September 2017, we sampled 26 wild cotton populations located along the coast of the states of Yucatan and Campeche (Fig. 2; Appendix S1), spanning most of this species' distribution in southeastern Mexico (see D'Eeckenbrugge and Lacape, 2014). Despite its relatively narrow coastal distribution range in the Yucatan Peninsula, abiotic conditions vary across the sampled sites. Mean annual precipitation varies up to 2.2-fold across sites, and although mean annual temperature varies considerably less (up to 1.3°C), mean maximum temperatures of the warmest month vary up to 3°C
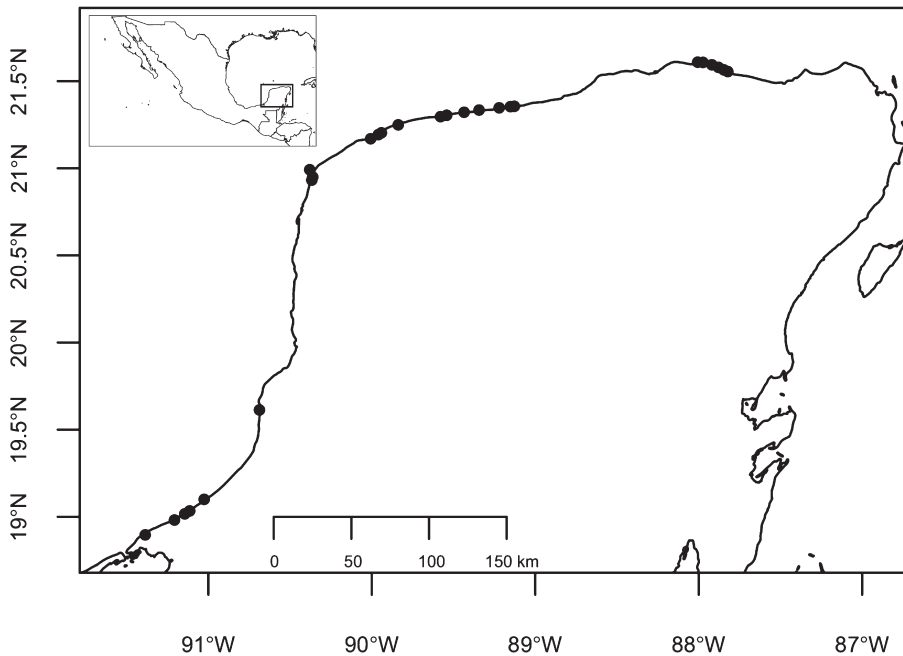
FIGURE 2. Map indicating the location of the wild cotton (*Gossypium hirsutum*) populations (*N* = 26) sampled along the coast of the Yucatan Peninsula (México).

across sites (Appendix S1). The distance between adjacent sites was at least 2 km, and at each site we sampled 7–8 plants that were separated by at least 2 m. Plants were similar in size and ranged in height from ca. 1 to 1.5 m. We performed sampling in late September, which represents the last third of the wet season when insect herbivore abundance is highest and most of the insect leaf damage has occurred. We measured plant cover for each individual by multiplying the plant length (longest distance from branch tip to tip) by its width (perpendicular to the length).

For each plant, we also selected a 1-m branch or two 0.5-m branches to visually estimate the percentage of area removed per leaf by chewing insects as 0%, 1–15%, 16–30%, 31–45%, 46–60%, or 61–75%. This methodological approach resulted in ca. 40–50 sampled leaves per plant. We used the midpoint of each category for each measurement and obtained the mean value across leaves per plant, which was then used for statistical analysis. Damage by leaf-chewing insects accounted for more than >90% of the recorded leaf herbivory; the remaining leaf damage was caused mostly by leaf miners (T. Quijano-Medina, personal observation). By sampling toward the end of the rainy season, our estimates of leaf herbivory represented a rough measure of cumulative leaf damage during previous months and therefore of overall damage levels during the growing season of the study. After assessing leaf herbivory, we collected 6–8 undamaged leaves from 4-5 plants per population for quantification of putative defense traits, specifically: density of gossypol glands, density of trichome needles (i.e., pubescence), and the concentration of phenolic compounds (see ahead). Sampling undamaged leaves was aimed at measuring constitutive levels of these traits, though some degree of systemic induction has to be assumed. We counted the number of glands (on the abaxial surface) and trichomes (adaxial surface) for two 0.20-cm² disks (excised from each of two leaves) using a stereoscopic microscope. In each case, values per disk were extrapolated to a 1-cm² area to obtain an estimate of the number of trichomes or glands per square centimeter. We then

obtained the mean of these extrapolated values for statistical analyses. Trichomes (hereafter pubescence) are stellate and typically have multiple ramifications or needles (up to six). Accordingly, we recorded the number of needles rather than the number of trichomes to provide a better estimate of the density of leaf pubescence.

Chemical analyses

Collected leaves for chemical analyses were stored in a cooler at 45°C (for sampling details, see Abdala-Roberts et al., 2019). Leaves were dried and ground to fine powder. Phenolic compounds were extracted using 20 mg of dry plant tissue (pooled leaves from each individual) with 1 mL of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation (Moreira et al., 2014). Supernates were then transferred to chromatographic vials for phenolic profiling using ultrahigh-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) and a 50 × 2.1 mm Acquity UPLC BEH C18 column (Waters, Milford, CT, USA) at 25°C. Solvents were water + 0.05% v/v formic acid (A) and acetonitrile + 0.05% v/v formic acid (B). The gradient program was set for a flow rate of 0.4 mL/min under the following conditions: 5–30% B for 6 min, 30–100% B for 2 min, holding at 100% B for 2 min followed by re-equilibration at 5% B for 2 min with an injection volume of 2 μL. The QTOF-MS was operated in MS^E negative mode over an *m/z* range of 85–1200 Da with capillary voltage at −2.5 kV, cone voltage at −25 V, source temperature at 120°C, desolvation gas temperature at 350°C, and desolvation gas flow at 800 L/h. The instrument was internally calibrated by infusing with solution of 400 ng/mL leucine-enkephaline at 15 μL/min through the Lock Spray probe. We tentatively identified phenolic compounds on the basis of their molecular formula (as determined from exact mass measurements), fragment ions, and comparison with available databases such as the Dictionary of Natural Products (Chapman and Hall, CRC Informa, London; version 20.2) or ReSpect for Phytochemicals (Sawada et al., 2012). The 24 different phenolics detected in cotton leaves comprised flavonoids, condensed tannins, hydrolysable tannins, and lignins. We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents by external calibration using calibration curves at 0.2, 0.8, 2, 5, and 20 μg/mL. We estimated total phenolics as the sum of flavonoids, lignins, condensed tannins and hydrolyzable tannins, and expressed concentrations of each group as micrograms per gram tissue (dry mass basis). Samples from one population were lost and therefore not included in the statistical analysis.

Geographic and climatic variables

Latitude and longitude of each cotton population were determined using a global positioning system device (Garmin, Olathe, KS, USA). To characterize the climatic conditions present at each site,

we used a subset of the BioClim climate variables (World Clim – global climate data, version 2; http://www.worldclim.org) based on the geographical coordinates of each population: BIO1 (annual mean temperature, °C), BIO4 (temperature seasonality, expressed as the standard deviation of temperature among months × 100), BIO5 (maximum temperature of the warmest month, °C), BIO6 (minimum temperature of the coldest month, °C), BIO12 (annual precipitation, mm), BIO13 (precipitation of the wettest month, mm), BIO14 (precipitation of the driest month, mm), BIO15 (precipitation seasonality, expressed as the standard deviation of precipitation across months) (Abdala-Roberts et al., 2016a; Moreira et al., 2018a). The procedures used to calculate these variables are based on interpolation methods described by Hijmans et al. (2005). WorldClim version 2 is based on data from 1970 to 2000, allowing us to test whether the recent climatic history of the sites correlated with current herbivory patterns. Such analyses imply a mismatch in the temporal scale of analysis for climate vs. herbivory data. This is important to consider, since current leaf damage patterns may be influenced by shorter-term variation in weather conditions (e.g., during the current or previous season), which may deviate from longer-term climate trends. Having said this, analyses based on short-term climatic data may produce patterns that are not representative of longer-term climatic variation across sites, which is problematic since longer-term climatic variation will influence broad-scale spatial variation in herbivory and arthropod communities, independently of short-term fluctuations. In sum, broad-scale spatial variation in arthropod abundance is expected to result from a combination of long- and short-term climatic variation whereby, we presume, effects of site variation in climatic history on herbivory act over and above effects of short-term climatic variation.

Statistical analyses

Climatic variables were previously summarized using principal component analyses (PCA) to simplify the evaluation of climatic effects and avoid inflating type I error due to multiple tests using individual climatic predictors (Abdala-Roberts et al., 2016a; Moreira et al., 2018a). The first principal component from the PCA of temperature variables explained 68% of the variance in the four temperature variables across populations ("PC temperature" hereafter), and was positively related to mean annual temperature and maximum temperature of the warmest month. Similarly, the first principal component from the PCA of precipitation variables explained 66% of the variance in the four precipitation variables across populations ("PC precipitation" hereafter), and was positively related to mean annual precipitation and precipitation of the wettest month. We used the standardized z-scores of the first PC in each case for the statistical analyses described below (structural equation models).

First, we assessed population variation in leaf traits and herbivory with general linear models using data at the plant level. These models tested for an effect of cotton population (fixed) on leaf pubescence, gossypol gland density, the concentration of phenolic compounds, and percentage leaf area consumed by insect leaf-chewers. Second, we conducted an analysis based on piecewise structural equation modelling (SEM) using population-level data (i.e., population means). We initially included all hypothesized associations between climatic factors, leaf defensive traits, and herbivory (following the causal model, Fig. 1), and then subsequently broke down this general model to test for specific

relationships involving underlying correlates of population variation in plant traits and herbivory. To be consistent with the causal model, we also included the effect of latitude on climatic variables to illustrate the direction of latitudinal variation in abiotic conditions (longitude was excluded as preliminary analyses indicated that this variable was not correlated to either cotton traits or herbivory). Direct and indirect effects of latitude on plant traits and herbivory were not included to simplify the model (and reduce parameter load) and, more importantly, because this predictor is a proxy of climatic variables and we sought to test the effects of underlying climatic variation acting upon and explaining latitudinal gradients in plant defenses and herbivory. Conventional SEM estimates the effect of predictors on response variables simultaneously, while in piecewise SEM the overall causal network is broken down into different independent linear regression models and then combined (Lefcheck, 2016). This approach allows specific assumptions to be incorporated into each of the regression models that were included in the SEM (Lefcheck, 2016). Direct effects in the SEM were estimated as standardized partial regression coefficients, whereas indirect effects were obtained by combining the specified coefficients for direct effects on both the predictor and the response. The significance of direct and indirect coefficients was assessed with t-tests. The goodness of fit of the general model was evaluated with a "test of direct separation" based on the Fisher's C-test. Relationships between response and significant predictors variables were tested using the partial residuals as extracted from the piecewise general model (Lefcheck, 2016). Gland density, leaf pubescence, and total phenolics were not significantly correlated (Pearson's $r < 0.27$, $P > 0.19$), indicating that collinearity did not affect results from this analysis. Although the climatic PCs were significantly correlated ($r = 0.78$, $P < 0.0001$), this correlation did not prevent detecting significant effects by these predictors (see Results), suggesting collinearity did not strongly influence the analysis.

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

RESULTS

We found significant variation among wild *G. hirsutum* populations in herbivory and in all the leaf defensive traits measured. Gossypol gland density varied up to 2-fold (302.50–616.09 glands/cm^2), leaf pubescence ranged from 0 to over 1600 needles/cm^2, and population variation in total phenolics varied up to 5.2-fold (702.34–3694.81 μg/g) (Table 1; Appendix S2). In addition, leaf herbivory varied up to 5.8-fold among populations (9.22–52.13% leaf area consumed) (Table 1; Appendix S2).

TABLE 1. Population mean (±SE) range for population variation in leaf traits in *G. hirsutum* from 26 populations in northern Yucatan and Campeche (SE Mexico). Data for phenolics for one population were excluded (see text). *F*- and *P*-values are from general linear models testing for an effect of population. Significant results (*P* < 0.05) are in bold.

Leaf trait	Mean range	F	P
Gland density (no./cm²)	302.50 ± 19.33 to 616.09 ± 63.71	4.58	**<0.001**
Leaf pubescence (no./cm²)	0 to 1681.88 ± 116.24	19.69	**<0.001**
Total phenolics (µg/g dry mass)	702.34 ± 101.06 to 3694.81 ± 145.14	9.75	**<0.001**
Herbivory (% leaf area consumed)	9.22 ± 0.87 to 52.13 ± 8.06	9.38	**<0.001**

Results from the piecewise SEM analysis showed expectedly significant negative associations between latitude and both temperature and precipitation (Fig. 3). Importantly, there were significant effects of climatic factors on cotton defenses and herbivory (Fig. 3), whereby precipitation negatively affected leaf pubescence (Figs. 3, 4A) and positively affected herbivory (Figs. 3, 4B), and temperature negatively affected herbivory (Figs. 3, 4C). There was also evidence that plant defenses were associated with herbivory; leaf pubescence (but not phenolics or gossypol glands) had a significant negative association with leaf damage (Figs. 3, 4D). Finally, there were no significant indirect effects of either temperature (coefficient = −0.46, *P* = 0.13) or precipitation (coefficient = 0.21, *P* = 0.28), i.e., via cotton traits, on herbivory.

DISCUSSION

There was substantial population variation in leaf defensive traits and herbivore damage for wild cotton along its coastal distribution on the Yucatan Peninsula. Our findings indicated that leaf pubescence, but not gossypol gland density or phenolics, was significantly negatively associated with population variation in insect leaf herbivory. We also found strong direct effects of temperature and precipitation on leaf herbivory whereby leaf damage on wild cotton was lower in warmer and drier sites (i.e., more arid habitats). In addition, precipitation was negatively associated with leaf pubescence. Finally, despite significant effects of climate on leaf pubescence and significant effects of this trait on herbivory, we found no evidence of indirect effects of climate (via pubescence) on leaf damage. Overall, these findings highlight concurrent bottom-up effects of leaf pubescence and climatic factors (precipitation and temperature) on insect leaf herbivory across populations of wild cotton.

Cotton traits correlated with spatial variation in herbivory

Our results showed no significant associations between gossypol gland density or phenolic compounds with insect leaf herbivory across wild cotton populations. These results run counter to previous work showing that gland density and concentrations of terpenoid (e.g., McAuslane and Alborn, 1997; Stipanovic et al., 2006; Optiz et al., 2008) and phenolic compounds (e.g., Mansour et al., 1997; Nix et al., 2017) play a role in resistance against insect herbivory in this plant species. Not all studies, however, support a role in direct resistance
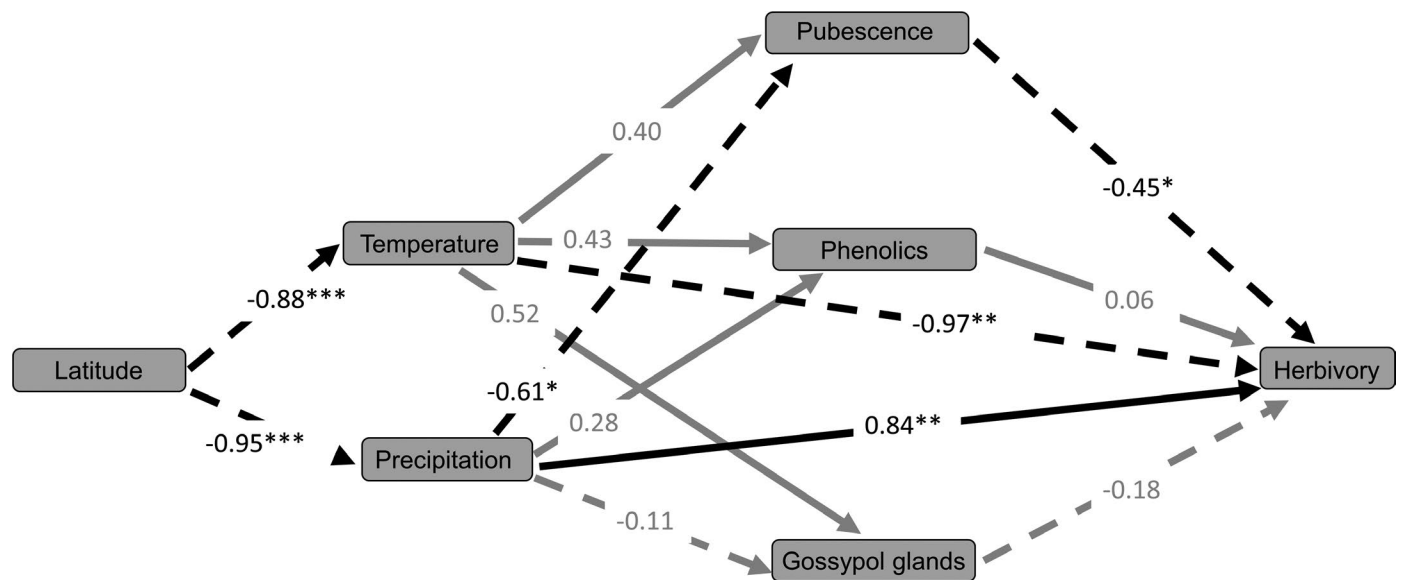

FIGURE 3. Diagram showing results from an analysis based on piecewise structural equation modelling testing for associations among latitude, climatic factors (temperature- and precipitation-related variables), leaf putative defensive traits (total phenolics, pubescence, gossypol gland density), and insect herbivory (percentage leaf area consumed) on *Gossypium hirsutum*. Values used in this analysis were population means (*N* = 26). Climatic variables represent *z*-score values from a principal components analysis summarizing a suite of variables associated to precipitation or temperature. Values next to each arrow are path coefficients (i.e., standardized regression coefficients). Continuous arrows indicate positive associations, whereas broken arrows indicate negative associations; we also tested for and did not find significant indirect effects of either temperature or precipitation on herbivory (arrows not shown for ease of visualization). This analysis also accounted for covariation between temperature and precipitation as well as between leaf defensive traits, but these estimates are not shown for ease of visualization. Significant (*P* < 0.05, **P* < 0.01, ***P* < 0.001) and nonsignificant (*P* > 0.05) path coefficients (text and arrows) are in black and grey, respectively. Explained variance: pubescence = 0.16; phenolics= 0.46; gossypol glands = 0.19; herbivory = 0.52. Fisher's *C* = 5.93, *P* = 0.431, AICc = 43.93.
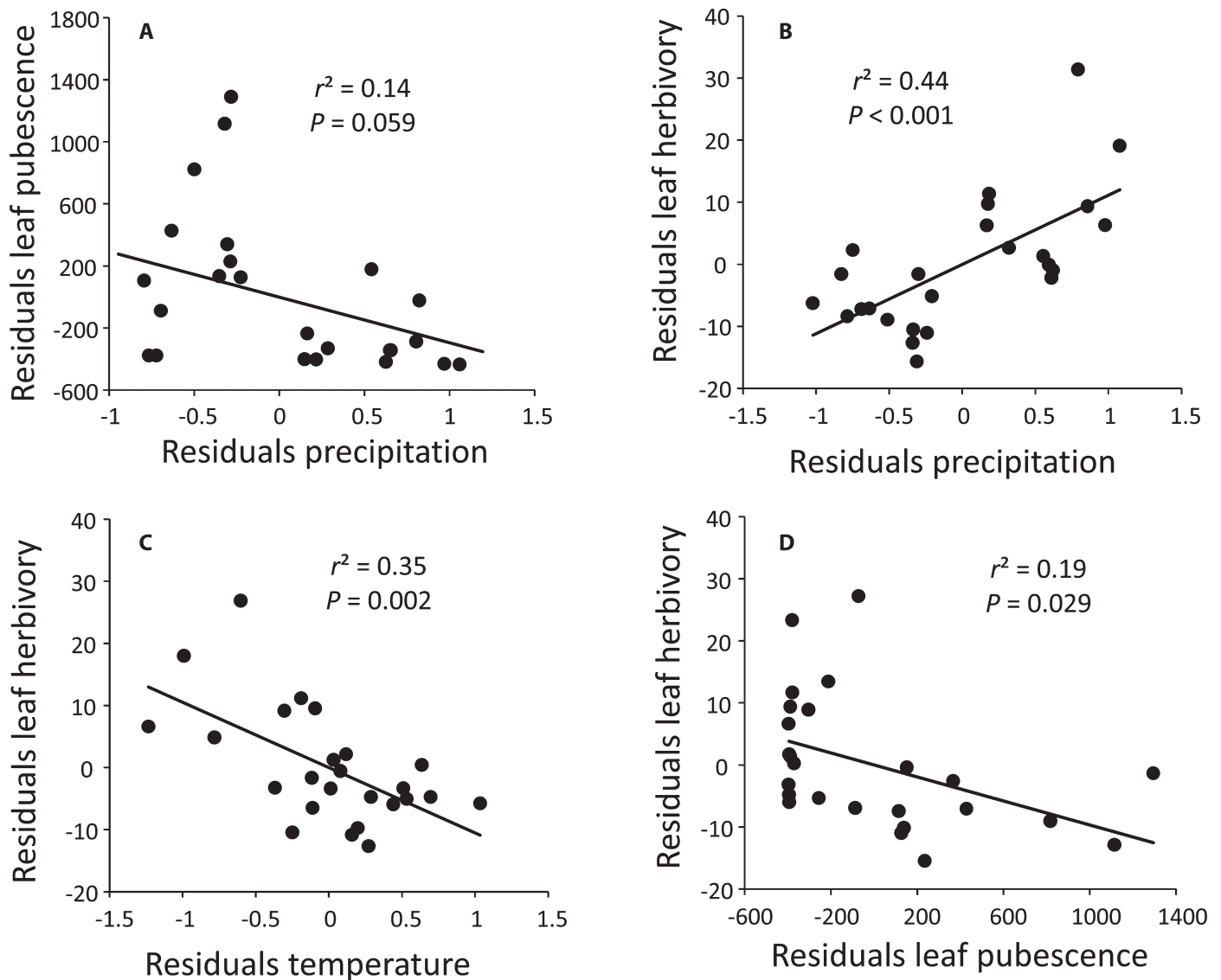
FIGURE 4. Partial residual plots from the piecewise structural equation analysis showing relationships between leaf pubescence (number of trichome needles/cm^2) and precipitation (A), and between insect leaf herbivory and precipitation (B), temperature (C), and leaf pubescence (D). Predicted relationships come from simple linear regressions and the slope of each relationship is the partial correlation coefficient (r^2) for the effect of the predictor on the response. Precipitation and temperature values used are z-scores from the first axis of two principal components analyses summarizing a suite of relevant precipitation- and temperature-related variables (see statistical analyses).

for these chemical traits (e.g., McAuslane and Alborn, 1998; Agrawal and Karban, 2000), suggesting that their defensive function is contingent on the herbivore species or guild under study or on abiotic conditions. Accordingly, our measurements of leaf herbivory included damage by different species of chewing insects (mainly grasshoppers, beetles, and caterpillars), and thus may have lacked the appropriate resolution to address whether pigment glands are associated with damage by specific herbivore guilds or species (e.g., dietary specialists). In addition, previous work has found that gland density and terpenoid production (per gland) both contribute to resistance against insect herbivores and should therefore be separated and individually measured (Opitz et al., 2008). Accordingly, future work with wild cotton populations must consider true terpenoid concentrations to fully test for effects of these compounds on herbivory.

Rather than gland density or phenolics, results pointed at leaf pubescence (i.e., trichome needle density) as the main leaf trait influencing herbivory across wild cotton populations. The defensive role of trichomes has long been studied in plants (Levin, 1973; Dalin et al., 2007), and their role in deterring herbivores and reducing insect damage in both wild (e.g. Agrawal, 1999; Valverde et al., 2001; Abdala-Roberts et al., 2016a) and cultivated (e.g., Wilkens et al., 1992; Kaplan et al., 2009) species has been reported. In *G. hirsutum*, however, the role of trichomes in direct defense has thus far received little attention, and the few studies available have involved cultivated varieties (Mahttews, 1989). The fact that trichomes were associated with our broad-spectrum measurements of leaf damage suggests a defensive role against a relatively diverse fauna of generalist insect herbivores feeding on wild cotton. The role of trichomes

as a driver of herbivory in wild cotton should, however, be taken with caution because our analyses are correlational in nature and population variation in the concentration of gossypol and other related terpenoids remains to be assessed (see above).

Effects of climate on spatial variation in leaf traits and herbivory

We found evidence for climatic control over wild cotton population variation in leaf pubescence. The density of trichome needles increased with aridity, suggesting that greater water stress and reduced resource availability increases investment in this putative defensive trait (following the resource availability hypothesis; Coley et al., 1985). Alternatively, leaf pubescence may play a role in plant tolerance to abiotic stress because leaf trichomes in many cases contribute to abiotic tolerance by influencing plant evapotranspiration and water-use efficiency (e.g., Hare and Elle, 2001). In addition, latitude was a significant predictor of pubescence after accounting for climatic factors in the model, suggesting that other unmeasured abiotic (or biotic) factors varying latitudinally influenced this leaf trait. Along the same lines, although not significant in the piecewise SEM (coefficient = 0.53 ± 0.29, $P = 0.09$), gland density also tended to increase with temperature. Terpenes have also been shown to play a role in mediating plant responses to abiotic stress, and recent work has found increasing leaf terpene concentrations under warmer and drier climatic conditions both within (Jamieson et al., 2012) and across plant populations (Pratt et al., 2014). Further work addressing the role of gossypol glands and trichomes in tolerance to abiotic stress in wild cotton is needed.

Climatic conditions had a strong direct influence on cotton population variation in insect herbivory. Leaf damage decreased with increasing aridity, which agrees with previous studies reporting stronger herbivore pressure with increasing precipitation (e.g., along latitudinal gradients; Schemske et al., 2009), whereas leaf damage decreased with temperature, counter to expectations. Recent studies conducted at broader spatial scales, including a greater range of variation in temperature (spanning tropical and temperate latitudes), have found unexpected associations between herbivory and temperature. For example, Zhang et al. (2016) reported a positive association between herbivory and temperature in the northern hemisphere and the inverse pattern for the southern hemisphere. These results suggest that the magnitude and sign of climate–herbivory associations vary across regions depending on the range, upper and lower limits of climatic conditions considered, and on biotic factors such as variation in plant and herbivore species composition and life history traits (Abdala-Roberts et al., 2016a; Zhang et al., 2016). In our case, a strong negative correlation with temperature was found despite relatively low variation across sites (range for mean annual temperature: 1.3°C), which agrees with our previous work also showing negative effects of temperature on population variation in insect seed predation for a perennial herb in the same region (Moreira et al., 2015; Abdala-Roberts et al., 2016a). Accordingly, the Yucatan Peninsula has a subtropical climate, and cotton populations found along the northern coast are exposed to more extreme temperatures during the dry and wet season (>35°C maximum monthly averages) relative to populations on the southwestern coast. This would presumably result in greater thermal stress on insects and therefore explain reduced herbivory rates at northern sites.

Overall, we found no evidence for indirect effects of temperature or precipitation on insect herbivory on wild cotton leaves, even though temperature significantly affected leaf pubescence, which significantly affected leaf herbivory. These findings run counter to recent work emphasizing the importance of bottom-up, plant-mediated effects of abiotic factors on consumers (Rosenblatt and Schmitz, 2016) and other work reporting significant indirect effects of climate on insect herbivore abundance, diversity, and damage (Pratt et al., 2017; Moreira et al., 2018a). However, indirect effects are frequently difficult to detect statistically, which may therefore require a larger population sample size, especially given the limited statistical power of our SEM. Thus, we caution on ruling out entirely plant-mediated indirect effects of climate on herbivory on wild cotton.

CONCLUSIONS

Overall, our results suggest an important role of putative leaf defensive traits and climatic conditions in shaping geographic patterns of insect leaf herbivory on wild cotton. Leaf pubescence and climatic factors appeared to exert particularly strong effects on insect leaf herbivory, and in the latter case, effects of climate were primarily direct (rather than indirect via leaf traits), though low power may have prevented the detection of indirect abiotic effects. Lacking from our analysis is an assessment of top-down control by natural enemies and its associated leaf traits in order to achieve a full assessment of biotic controls over geographic variation in herbivory on wild cotton (e.g., see Björkman et al., 2011). Accordingly, Further work should include surveys of predator abundance, particularly ants and spiders, which are the dominant groups of predators on wild cotton, and assess traits such as extrafloral nectar and volatile compounds that potentially mediate recruitment of these natural enemies. Overall, our results imply that a robust understanding of bottom-up controls over geographic variation in herbivory requires addressing the simultaneous and independent effects of plant defensive traits and climatic factors. Combining observational approaches such as the present study with experimental manipulations of herbivory, predators, and abiotic factors will yield the greatest insight into the mechanisms governing plant–herbivore interactions.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Geographical coordinates (decimal degrees) and climatic characteristics (mean annual temperature in °C and

mean annual precipitation in mm for 26 populations of wild cotton (*Gossypium hirsutum*) sampled in the states of Yucatan and Campeche (SE Mexico).

APPENDIX S2. Wild cotton (*Gossypium hirsutum*) population variation in leaf traits (gossypol glands/centimeter, trichome needles/centimeter, and total phenolics m/mg), insect leaf herbivory (percentage leaf area removed or damaged), and abundance of leaf-chewing insects (number of insects per plant).

LITERATURE CITED

Abdala-Roberts, L., and K. A. Mooney. 2015. Plant and herbivore evolution within the trophic sandwich. *In* T. Hanley and K. La Pierre [eds.], Trophic interactions: bottom-up and top-down interactions in aquatic and terrestrial ecosystems, 340–364. Cambridge University Press, Cambridge, UK.

Abdala-Roberts, L., X. Moreira, S. Rasmann, V. Parra-Tabla, and K. A. Mooney. 2016a. Test of biotic and abiotic correlates of latitudinal variation in plant defenses in the perennial herb *Ruellia nudiflora*. *Journal of Ecology* 104: 580–590.

Abdala-Roberts, L., B. Pérez-Niño, X. Moreira, V. Parra-Tabla, L. Grandi, G. Glauser, B. Benrey, and T. C. J. Turlings. 2019. Effects of early-season insect herbivory on subsequent pathogen infection and ant abundance on wild cotton (*Gossypium hirsutum*). *Journal of Ecology* 107: 1518–1529.

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecology* 80: 1713–1723.

Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defense. *Functional Ecology* 25: 420–432.

Agrawal, A., and R. Karban. 2000. Specificity of constitutive and induced resistance: pigment glands influence mites and caterpillars on cotton plants. *Entomologia Experimentalis et Applicata* 96: 39–49.

Anstett, D. N., K. A. Nunes, C. Baskett, and P. M. Kotanen. 2016. Sources of controversy surrounding latitudinal patterns in herbivory and defense. *Trends in Ecology and Evolution* 31: 789–802.

Björkman, C., Å. Berggren, and H. Bylund. 2011. Causes behind insect folivory patterns in latitudinal gradients. *Journal of Ecology* 99: 367–369.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230: 895–899.

Dalin, P., J. Ågren, C. Björkman, P. Huttunen, and K. Kärkäinen. 2008. Leaf Trichome Formation and Plant Resistance to Herbivory. pp. 89–105. *In* A. Schaller [ed.], Induced Plant Responses to Herbivory. Springer, Dordrecht, the Netherlands.

D'Eeckenbrugge, G. C., and J. M. Lacape. 2014. Distribution and differentiation of wild, feral, and cultivated populations of perennial upland cotton (*Gossypium hirsutum* L.) in Mesoamerica and the Caribbean. *PLoS ONE* 9: e107458.

Endara, M. J., and P. D. Coley. 2011. The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology* 25: 389–398.

Gripenberg, S., and T. Roslin. 2007. Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos* 116: 181–188.

Hagenbucher, S., D. M. Olson, J. R. Ruberson, F. L. Wäckers, and J. Romeis. 2013. Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Critical Reviews in Plant Sciences* 32: 458–482.

Hahn, P. G., and J. L. Maron. 2016. A framework for predicting intraspecific variation in plant defense. *Trends in Ecology and Evolution* 31: 646–656.

Hare, J. D., and E. Elle. 2001. Geographic variation in the frequencies of trichome phenotypes of *Datura wrightii* and correlations with annual water deficit. *Madroño* 48: 33–37.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73: 724–732.

Jamieson, M., A. M. Trowbridge, K. F. Raffa, and R. L. Lindroth. 2012. Consequences of climate warming and altered precipitation patterns for plant–insect and multitrophic interactions. *Plant Physiology* 160: 1719–1727.

Johnson, M. T. J., and S. Rasmann. 2011. The latitudinal herbivory-defence hypothesis takes a detour on the map. *New Phytologist* 191: 589–592.

Kaplan, I., G. P. Dively, and R. F. Denno. 2009. The costs of anti-herbivore defense traits in agricultural crop plants: a case study involving leafhoppers and trichomes. *Ecological Applications* 19: 864–872.

Lefcheck, J. S. 2016. piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7: 573–579.

Levin, D. A. 1973. The role of trichomes in plant defence. *Quarterly Review of Biology* 48: 3–15.

Mansour, M. H., N. M. Zohdy, S. E. ElGengaihi, and A. E. Amr. 1997. The relationship between tannins concentration in some cotton varieties and susceptibility to piercing sucking insects. *Journal of Applied Entomology* 121: 321–325.

Matthews, G. A. 1989. Cotton insect pests and their management. Longman, Harlow, UK; Wiley, NY, NY, USA.

McAuslane, H. J., and H. T. Alborn. 1997. Systemic induction of terpenoid aldehydes in cotton pigment glands by feeding of larval *Spodoptera exigua*. *Journal of Chemical Ecology* 23: 2861–2879.

McAuslane, H. J., and H. T. Alborn. 1998. Systemic induction of allelochemicals in glanded and glandless isogenic cotton by *Spodoptera exigua* feeding. *Journal of Chemical Ecology* 24: 399–416.

McCall, P. J., T. C. Turlings, J. Loughrin, A. T. Proveaux, and J. H. Tumlinson. 1994. Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *Journal of Chemical Ecology* 20: 3039–3050.

Moles, A. T., S. O. Bonser, A. G. B. Poore, I. R. Wallis, and W. J. Foley. 2011. Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology* 25: 380–388.

Moreira, X., K. A. Mooney, S. Rasmann, W. K. Petry, A. Carrillo-Gavilán, R. Zas, and L. Sampedro. 2014. Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecology Letters* 17: 537–546.

Moreira, X., L. Abdala-Roberts, V. Parra-Tabla, and K. A. Mooney. 2015. Latitudinal variation in herbivory: influences of climatic drivers, herbivore identity, and natural enemies. *Oikos* 124: 1444–1452.

Moreira, X., B. Castagneyrol, L. Abdala-Roberts, J. C. Berny-Mier y Terán, B. G. Timmermans, H. H. Bruun, F. Covelo, et al. 2018a. Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory. *Ecography* 41: 1124–1134.

Moreira, X., W. Petry, K. A. Mooney, S. Rassman, and L. Abdala-Roberts. 2018b. Elevational gradients in plant defense and herbivory: recent advances in the field and prospects for future research. *Ecography* 41: 1485–1496.

Nix, A., C. Paull, and M. Colgrave. 2017. Flavonoid profile of the cotton plant, *Gossypium hirsutum*: a review. *Plants* 6: 43.

Oosterhuis, D. M., and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant. *In* W. C. W. Smith and J. T. Cothren [eds.], Cotton: origin, history, technology and production, 175–206. John Wiley, NY, NY, USA.

Opitz, S., G. Kunert, and J. Gershenzon. 2008. Increased terpenoid accumulation in cotton (*Gossypium hirsutum*) foliage is a general wound response. *Journal of Chemical Ecology* 34: 508–522.

Pearse, I. S., and A. L. Hipp. 2012. Global patterns of leaf defenses in oak species. *Evolution* 66: 2272–2286.

Pratt, J. D., K. Keefover-Ring, L. Y. Liu, and K. A. Mooney. 2014. Genetically based latitudinal variation in *Artemisia californica* secondary chemistry. *Oikos* 123: 953–963.

Pratt, J. D., A. Datu, T. Tran, D. C. Sheng, and K. A. Mooney. 2017. Genetically based latitudinal clines in *Artemisia californica* drive parallel clines in arthropod communities. *Ecology* 98: 79–91.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org/.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. *In* G. A. Rosenthal and D. H. Janzen [eds.], Herbivores: their interaction with secondary plant metabolites, 4–54. Academic Press, NY, NY, USA.

Rosenblatt, A. E., and O. J. Schmitz. 2016. Climate change, nutrition, and bottom-up and top-down food web processes. *Trends in Ecology and Evolution* 31: 965–975.

Rudgers, J. A., S. Y. Strauss, and J. F. Wendel. 2004. Trade-offs among anti-herbivore resistance traits: insights from Gossypieae (Malvaceae). *American Journal of Botany* 91: 871–880.

Sawada, Y., R. Nakabayashi, Y. Yamada, M. Suzuki, M. Sato, A. Sakata, K. Akiyama, et al. 2012. RIKEN tandem mass spectral database (ReSpect) for phytochemicals: a plant-specific MS/MS-based data resource and database. *Phytochemistry* 82: 38–45.

Schemske, D. W., G. G. Mittelbach, H. V. Cornell, J. M. Sobel, and K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology and Systematics* 40: 245–269.

Stipanovic, R. D., J. D. Lopez, M. K. Dowd, L. S. Puckhaber, and S. E. Duke. 2006. Effect of racemic and (+)- and (−)-gossypol on the survival and development of *Helicoverpa zea* larvae. *Journal of Chemical Ecology* 32: 959–968.

Valverde, P. L., J. Fornoni, and J. Núñez-Farfán. 2001. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium. Journal of Evolutionary Biology* 14: 424–432.

Wäckers, F. L., and T. M. Bezemer. 2003. Root herbivory induces an aboveground indirect defence. *Ecology Letters* 6: 9–12.

Wendel, J. F., C. L. Brubaker, and A. E. Percival. 1992. Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. *American Journal of Botany* 79: 1291–1310.

Wilkens, R. T., G. O. Shea, S. Halbreich, and N. E. Stamp. 1996. Resource availability and the trichome defenses of tomato plants. *Oecologia* 106: 181–191.

Zhang, S., Y. Zhang, and K. Ma. 2016. Latitudinal variation in herbivory: hemispheric asymmetries and the role of climatic drivers. *Journal of Ecology* 104: 1089–1095.