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2 **Effects of variability in the amount and dispersion of within-plant**
3 **herbivory on resistance- and tolerance-related responses in wild cotton**

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22 **Main Conclusion**

23 Variability in caterpillar damage to wild cotton leaves differentially shapes resistance- and tolerance-
24 related responses; dispersed damage induces more extrafloral nectar production, and concentrated
25 damage more strongly drives reductions in regrowth.

26

27 **Abstract**

28 Herbivory triggers complex induced defensive responses in plants that may vary depending on the
29 amount and dispersion of damage, the latter has received much less attention. We examined how wild
30 cotton (*Gossypium hirsutum*) responds to different amounts of herbivory and how it is dispersed among
31 the leaves (concentrated versus dispersed) using the specialist caterpillar *Alabama argillacea*. In a
32 greenhouse experiment, plants underwent different herbivory treatments: no damage, low herbivory
33 (two caterpillars, one on each of two leaves), high concentrated herbivory (four caterpillars, two on
34 each of two leaves), and high dispersed herbivory (four caterpillars, each on one of four leaves). We
35 measured extrafloral nectar (EFN), an indirect defense trait, and phenolic production, a direct chemical
36 defense, as well as regrowth capacity (new leaf biomass two months after herbivory) as a tolerance
37 proxy. To distinguish between local and systemic defense induction, we measured resistance-related
38 responses on damaged and undamaged leaves. EFN was significantly and more consistently induced in
39 damaged leaves (i.e., stronger local induction) in response to high dispersed damage. Phenolic
40 compounds were not affected by any of the damage treatments. Additionally, wild cotton fully
41 compensated for damage in terms of new leaf biomass under all herbivory treatments, except after high
42 concentrated damage, suggesting higher costs incurred by this type of herbivory. These results highlight
43 the importance of studying plant-induced defenses under varying herbivory conditions and suggest
44 opposing effects on resistance-related (EFN, indirect) versus tolerance-related induced responses,
45 which are known to affect tri-trophic interactions associated with wild cotton.

46

47 **Keywords:** *Gossypium hirsutum*, extrafloral nectar, *Alabama argillacea*, damage uniformity, phenolic
48 compounds, specialist herbivore

49 **Introduction**

50 Herbivory has profound ecological and evolutionary impacts on plants (Strauss and Agrawal 1999;
51 Karban 2011; Fritz and Simms 2012; Kant et al. 2015; Archibald et al. 2019; Adedayo et al. 2025). To
52 mitigate the adverse effects, plants have evolved a wide array of defenses to either resist or tolerate
53 herbivory (Leimu and Koricheva 2006, Núñez-Farfán et al. 2007; Fontes-Puebla and Bernal 2020).
54 Direct resistance includes structural traits such as trichomes and chemical deterrents, including
55 repellent or toxic secondary metabolites (Mauricio 2000; Núñez-Farfán et al. 2007; Endara et al 2023).
56 Indirect resistance includes traits that attract natural enemies of herbivores by offering housing (e.g.,
57 domatia), signals that indicate the presence of herbivorous prey (volatile organic compounds), or food
58 rewards such as extrafloral nectar (EFN) (Heil 2008; Kessler 2017). Tolerance, in contrast, is the plant's
59 ability to withstand or recover from herbivory, often through compensatory growth or resource
60 reallocation, thereby minimizing fitness losses (Mauricio 2000; Núñez-Farfán et al. 2007; Salgado-
61 Luarte et al. 2022; Liñán-Vigo and Núñez-Farfán 2024). Both resistance and tolerance strategies
62 involve induced responses and resource reallocation, and they are often correlated (Strauss and
63 Agrawal 1999; Leimu and Koricheva 2006; Oduor et al. 2011; Fornoni 2011) although this is not
64 always the case (Leimu and Koricheva 2006; Carmona and Fornoni 2012; Kariñho-Betancour and
65 Núñez-Farfán 2015).

66 The amount of herbivory is a key driver of plant resistance and tolerance strategies (War et al.
67 2012). Induced resistance mechanisms, such as the production of chemical defenses, are often
68 herbivore dose-dependent, with higher damage levels frequently triggering stronger induced responses

(Underwood 2000, 2010; Paniagua Montoya et al. 2024). In some cases, the responses are non-linear. For example, EFN sugar concentration, an indirect defense, may peak at intermediate damage levels and not increase upon further damage (Pimenta et al. 2025). Similar non-linear patterns have been reported for secondary metabolites (Karban and Baldwin 1997; Karban 2011; Eisenring et al. 2017). On the tolerance side, plants subjected to higher levels of damage are often unable to compensate fully, resulting in reduced growth or reproduction (Mundim et al. 2012; Tito et al. 2016; Quijano-Medina et al. 2019). These findings suggest that the costs of herbivory increase as damage levels exceed the plant's capacity to reallocate resources for subsequent growth and reproduction (Núñez-Farfán et al. 2007). In some cases, overcompensation with increasing damage has been found for both vegetative and reproductive tissues (Paige 2018; Garcia and Eubanks 2019; Gols 2025). Understanding these damage-dependent responses is crucial for predicting plant fitness outcomes, the evolution of plant defensive strategies, and the coevolutionary dynamics between plants and herbivores.

The effects of varying amounts of herbivore damage on plants have been frequently studied, yet spatial patterning of damage among the leaves, i.e. how even (disperse) or uneven (concentrated) is distributed the damage, can be equally important in determining herbivory effects on plant responses and performance (The Herbivory Variability Network et al. 2023; Wetzel et al. 2023). The modular structure of plants and constraints on resource exchange among modules explain why they commonly better tolerate dispersed herbivory than concentrated herbivory (Marquis 1992, 1996; Mauricio et al. 1993). Indeed, when damage is concentrated, constraints to resource redistribution impair the recovery of affected modules, thereby reducing overall plant growth and reproduction (a phenomenon known as subcompensation). Less is known about the effects of variable degrees and patterns of herbivory on induced defense responses (but see: Ohnmeiss et al. 1997; Paniagua Montoya et al. 2024; Martín-Cacheda et al. 2025). Direct vascular connections that enable defense induction in undamaged leaves are linked to damaged ones via the exchange of metabolites as well as reallocation of resources among

93 modules (Schittko and Baldwin 2003; Ferrieri et al. 2015; Eisenring et al. 2017). However, how
 94 different degrees of herbivory affect plant defense induction may differ for different plant traits and be
 95 dependent on the relative importance of systemic vs. local induction responses. On one hand, traits that
 96 are influenced by systemic responses are expected to be affected by physiological constraints that limit
 97 exchanges between different tissues (Schultz et al. 2013; Ferrieri et al. 2015). On the other hand, traits
 98 primarily induced locally may not exhibit such constraints, at least not in the short term, as their
 99 expression does not rely on inter-module resource reallocation; they occur within modules or parts of
 100 them (Marquis 1996). If this is the case, it is presumed that locally induced defenses are more strongly
 101 induced by concentrated damage in order to promote herbivore movement to other plant parts or
 102 neighboring plants (Marquis 1996). To date, very few studies have simultaneously tested how intensity
 103 and within-plant dispersion of herbivory affect resistance- and tolerance-related plant-induced
 104 responses. We here address this gap by studying resistance- and tolerance-related induced responses to
 105 herbivore damage in wild cotton (*Gossypium hirsutum* L.). Wild cotton occurs naturally in populations
 106 along the coast of northern Yucatan (Mexico), its likely center of origin and domestication (Wendel and
 107 Grover 2015). It is a myrmecophytic shrub that exhibits highly inducible direct (e.g., secondary
 108 metabolites) and indirect (e.g., EFN that attracts ants) resistance traits (Quijano-Medina et al. 2021;
 109 Briones-May et al. 2023; Mamin et al. 2025). It also appears to have tolerance mechanisms that
 110 mediate regrowth capacity in response to leaf damage, with a high amount of damage (e.g., 50% of
 111 total leaf area removal) leading to subcompensation (Quijano-Medina et al. 2019).

112 In our study, we inflicted different amounts and patterns of herbivore damage using the cotton
 113 specialist *Alabama argillacea*, which commonly occurs in the Yucatan cotton populations. In these
 114 populations, wild cotton plants exhibit a high degree of variability in the amount and distribution
 115 patterns of herbivore damage (Abdala-Roberts et al. 2019a), which has been shown to influence the
 116 induction of certain chemical defenses such as volatile organic compounds (Martín-Cacheda et al.

116 2025). In a greenhouse experiment, we exposed four-month-old plants to *A. argillacea* herbivory,
 117 varying the amount of damage as well as its within-plant dispersion. We then measured the levels of
 118 phenolic compounds and EFN, as well as leaf regrowth capacity. We hypothesized that plants would be
 119 better able to tolerate low than high damage (Quijano-Medina et al. 2019), more dispersed than
 120 concentrated damage, and, following from this, more strongly induce resistance-related traits in
 121 response to high vs. low damage and in response to concentrated vs. dispersed damage to mitigate the
 122 higher costs of high and concentrated damage. We further expected that resistance-related responses
 123 would be different for the two studied traits: EFN is largely induced locally (Abdala-Roberts et al.
 124 2019b; Briones-May et al. 2023); therefore, it is less limited by physiological constraints and expected
 125 to be more strongly induced under concentrated damage. In contrast, phenolic compounds are induced
 126 systemically (Abdala-Roberts et al. 2019b), which may constrain their induction under concentrated
 127 damage.

129 **Methods**

130 **Study species**

131 Wild populations of *Gossypium hirsutum* L., commonly known as upland cotton, are distributed across
 132 southern Mexico, Central America, and the Caribbean, and are particularly common in the Yucatan
 133 Peninsula (Mexico) (Coppens d'Eeckenbrugge and Lacape 2014; Wendel and Grover 2015). In the
 134 Yucatan, populations are typically found in coastal shrublands, where leaf damage is primarily caused
 135 by grasshoppers and caterpillars (Abdala-Roberts et al. 2019a). Damage from these chewing insects is
 136 highly variable, showing up to a 3.5-fold difference among individuals within populations and up to a
 137 threefold difference in variability within plants (i.e., dispersion of damage among leaves on the same
 138 plant) (Abdala-Roberts et al. 2019a). Among the herbivores, the specialist caterpillar *A. argillacea* is
 139 particularly common and exhibits substantial variation in abundance across sites, ranging from rare to
 140 outbreak levels (L. Abdala-Roberts pers. obs.). Wild cotton defends itself against insect herbivores
 141 directly as well as indirectly (Hagenbucher et al. 2013). Direct defenses include toxic terpenoid
 142 aldehydes stored in pigmented glands (e.g., gossypol, heliocides; Opitz et al. 2008; Eisenring et al.
 143 2017; Mamin et al. 2023), volatile organic compounds (also involved in plant-plant signaling; Grandi et
 144 al. 2024; Mamin et al. 2025), and phenolic compounds (Nix et al. 2017; Quijano-Medina et al. 2021).
 145 Phenolic compounds are particularly strongly induced in response to both natural and artificial
 146 herbivory in this species (e.g., Abdala-Roberts et al. 2019b; Quijano-Medina et al. 2021) and contribute
 147 to herbivore resistance (Nix et al. 2017; Quijano-Medina et al. 2024). In addition, wild cotton produces
 148 EFN in specialized nectaries, primarily located along the mid-vein on the ventral side of leaves, and
 149 EFN is also highly inducible in response to leaf damage (Abdala-Roberts et al. 2019b, Briones-May et
 150 al. 2023). However, we still know very little about the relative effects of herbivory amount and damage

151 dispersion on the induction of secondary compounds such as phenolics, and on indirect defenses such
152 as EFN (but see Martín-Cacheda et al. 2025).

153 **Plant material and propagation**

154 In 2023, seeds were collected from seven adult *G. hirsutum* plants (hereafter referred to as
155 “genotypes”) at a coastal scrubland site near Sisal, Yucatan, Mexico (21°11'45.3" N; 89°57'18.0" W). In
156 February 2024, seeds were germinated, and seedlings were transplanted into 2-liter bags filled with a
157 1:2:1 mix of sand, native forest soil, and perlite. Plants were grown in a greenhouse at the Campus de
158 Ciencias Biológicas y Agropecuarias (CCBA), Universidad Autónoma de Yucatán (20°52'00.6" N;
159 89°37'29.5" W), watered every other day with 300 ml of water, and used for experiments when they
160 were four months old.

161 **Experimental Design**

162 A total of 100 plants were placed in a greenhouse at the CCBA, spaced 90 cm apart. Plants were
163 subjected to one of four herbivory damage treatments, with genotypes approximately evenly distributed
164 across treatments. Damage was applied using clip cages containing a single third- or fourth-instar *A.*
165 *argillacea* caterpillar. The treatments were as follows: (a) control: two clip cages with no larvae (one on
166 leaf 2 and one on leaf 4, counting from the apical meristem) to control for clip cage effects; (b) low
167 herbivory: two larvae, one on each of two leaves (leaves 2 and 4); (c) high concentrated herbivory: four
168 larvae in total, with two larvae per leaf on leaves 2 and 4 (each larva placed in a separate clip cage,
169 with cages on opposite sides of each leaf); and (d) high dispersed herbivory: four larvae total, one on
170 each of four leaves (leaves 2, 3, 4, and 5). Larvae were placed on the plants for two consecutive nights
171 (from 18:00 to 6:00 each day). We use nighttime to expose plants to larvae because the high daytime
172 temperatures inside the greenhouse can cause the larvae to die. Under this setup, plants of the low-
173 damage treatment experienced approximately half the damage compared to the high-damage
174 treatments. Whereas both high-damage treatments resulted in similar total leaf area consumed, allowing

us to test for the effects of damage dispersion while holding overall damage constant. To verify that these treatments achieved the intended herbivory levels, we photographed leaves 2–5 of all plants after the treatment period. Using ImageJ (version 1.59, Schneider et al. 2012), we measured total and damaged leaf area and calculated the percentage of herbivory from these four leaves. As expected, the two high-damage treatments did not differ significantly in total damage in leaves 2-5, but both resulted in approximately twice the damage observed in the low-damage treatment (Fig. S1, supplementary material).

EFN volume and sugar quantification

EFN is rapidly induced after herbivory (within 12–48 h; Wäckers and Bonifay 2004; Abdala-Roberts et al. 2019b; Briones-May et al. 2023). Accordingly, we collected EFN from 98 plants the morning following the second night of larval exposure, between 6:00 and 8:00, using 5-μL capillary tubes (micropipettes Blaubrand® intraMARK, white color code, Germany). For each plant, separate samples were taken from leaves 1 and 2 (damaged and undamaged, respectively) to distinguish between local and systemic induced responses (Abdala-Roberts et al. 2019b; Briones-May et al. 2023). All nectaries were reset 24 h before sampling by removing existing nectar with cotton wool. After measuring EFN volume with capillary tubes, we assessed sugar concentration using a refractometer (Atago Master, Germany), recorded in ° Brix. Total sugar production per leaf was then estimated by multiplying nectar volume by sugar concentration, assuming 1 ° Brix equals 1 g of sugar per 100 g of solution (Kimball 1991).

194

Quantification of phenolic compounds

All plants remained in the greenhouse after EFN sampling, and a subsample of 50 plants was used for phenolic analysis (randomly selected within each treatment and evenly distributed across treatments

198 and genotypes). These plants were sampled one week after the herbivory treatments by harvesting
 199 leaves 1 and 2 (the youngest leaves). Collected leaves were immediately dried at 40 °C for three days
 200 (Moreira et al. 2025). This sampling procedure was chosen because the induction of phenolic
 201 compounds induced responses is typically slower, with significant effects on preexisting leaves
 202 reported to emerge between 72 h and one week after damage (Abdala-Roberts et al. 2019b; Quijano-
 203 Medina et al. 2023). Total phenolic content was determined using the Folin–Ciocalteu colorimetric
 204 method (Adegbusi et al. 2022). A standard curve was constructed using tannic acid, and results are
 205 reported as milligrams of tannic acid equivalents (TAE) per g of dry weight (DW).

206

207 **Regrowth responses**

208 The remaining 50 plants that were not used for phenolic quantification were assessed for their regrowth
 209 capacity as a proxy for tolerance to herbivory. These plants were kept in the greenhouse for an
 210 additional two months, after which all newly produced leaves (post-treatment) were harvested and
 211 dried at 45 °C for one week. The total DW of the new leaves was then measured using an analytical
 212 balance (± 0.1 mg, FA2004, Liberty, USA) to evaluate treatment effects on regrowth in terms of leaf
 213 biomass (Quijano-Medina et al. 2019).

214

215 **Statistical analyses**

216 We tested the effect of damage treatment (fixed, four levels) on nectar volume and total sugar
 217 production using generalized linear mixed models (GLMMs) with a Tweedie distribution and a log link
 218 function. The Tweedie distribution provided a good fit for the EFN data, which exhibited a right-
 219 skewed distribution with zeros (Hasan and Dunn 2015). In addition, we employed linear mixed models
 220 (LMMs) for phenolic compound concentrations and the DW of new leaves. All models included plant

height as a covariate to account for variability in plant size that could influence induced responses and regrowth capacity, and plant genotype (source mother plant) as a random factor to account for background variation due to genetic or maternal effects.

We performed all statistical analyses using R 4.4.2. (R Core Team 2024) with the following packages: “glmmTMB” to fit GLMMs (Brooks et al. 2017), “lme4” to fit LMMs (Bates et al. 2015), “DHARMa” to evaluate model fit (Hartig 2024), and “car” to test the main effects in GLMMs using Wald χ^2 tests (Fox and Weisberg 2019). In addition, we used “lmerTest” to compute F- and P-values for the main effects in LMMs (Kuznetsova et al. 2017), “emmeans” to perform Tukey pairwise contrasts between treatment groups and to extract back-transformed estimated marginal means (EMMs) with 95% confidence intervals (CIs) from the models (Lenth 2024), and “ggplot2” to visualize the results (Wickham 2016). Throughout the text, results are reported as EMM [2.5% lower CI, 97.5% upper CI].

Results

Resistance-related induced defenses

Extrafloral nectar. We found no significant effect of the damage treatment on EFN volume or sugar content produced by leaf 1 (undamaged, i.e., systemic induction) (Table 1; Fig. 1a, b). However, there was a significant effect of the damage treatment on both nectar volume and concentration for leaf 2 (damaged, i.e., local induction) (Table 1). This effect was most evident for the volume produced after high dispersed damage treatment (0.598 μ L [0.408, 0.876]), followed by the low damage treatment (0.423 μ L [0.281, 0.636]), which did not differ significantly from the former (Fig. 1c). These two treatments differed significantly from both the control (0.17 μ L [0.105, 0.276]) and the high concentrated treatment (0.144 μ L [0.084, 0.244]), with the latter not differing from the control (Fig. 1c). Regarding leaf 2 sugar content, high dispersed damage produced the highest mean value (0.375 μ g

244 [0.292, 0.482]) and differed significantly from all other groups, including the control (0.158 μg [0.11,
245 0.228]) (Fig. 1d). The other damage treatments (low damage: 0.214 μg [0.154, 0.297]; high
246 concentrated damage: 0.139 μg [0.091, 0.211]) did not differ from each other or the control (Fig. 1d).
247 *Phenolic content*. Although there was a trend toward higher induction under the high dispersed damage
248 treatment (Fig. 2), we found no significant effect of the damage treatment on phenolic content for either
249 leaf 1 or leaf 2 (Table 1).

250

251 **Tolerance-related defensive responses**

252 We found a significant effect of the damage treatment on the DW of new leaves produced after damage
253 (Table 1). Group comparisons showed that plants subjected to high concentrated damage (1.38 g [1.16,
254 1.6]) were the only treatment group with a significantly lower mean DW than control plants (1.87 g
255 [1.67, 2.07]) (Table 1, Fig. 2). Despite a trend, high concentrated damage did not differ significantly
256 from the other damage treatments (low damage: 1.6 g [1.4, 1.8]; high dispersed damage: 1.6 g [1.4,
257 1.8]), and these two treatments also did not differ from each other (Fig. 3).

258

259 **Discussion**

260 Our study shows that the characteristics of herbivory, namely its amount and its spatial dispersion
261 among the leaves, differentially affect resistance- and tolerance-related defensive responses in wild
262 cotton. Consistent with our predictions, we found that regrowth capacity was mainly compromised
263 under concentrated damage, suggesting higher costs associated with this herbivory pattern and
264 highlighting the role of within-plant herbivore variability as a key driver of tolerance responses in
265 cotton. Contrary to our expectations, EFN induction, particularly sugar content, was more strongly
266 triggered by dispersed damage (with a similar trend for EFN volume, albeit comparable to that

observed under low damage), while phenolic content was not significantly increased in response to any damage treatment. Therefore, although concentrated damage may have a greater negative impact on fitness, it did not lead to stronger induction of resistance-related traits that might mitigate these costs. Overall, these findings emphasize the importance of considering variation in herbivory and accounting for spatial patterns of damage when investigating plant defensive strategies and their associated induced responses. The significant reduction in new leaf DW observed in plants subjected to concentrated damage supports the hypothesis that concentrated herbivory imposes greater costs, possibly through physiological constraints, than dispersed damage. This finding aligns with previous research on the effects of plant modularity on plant tolerance responses, which emphasizes constraints on resource redistribution and regrowth capacity (Marquis 1992; Mauricio et al. 1993; Wetzel et al. 2023). Although the four-month-old cotton plants used in our experiment had not yet started branching, our results suggest that these constraints operate even among leaves within the same stem. Because our concentrated herbivory treatment targeted alternate leaves, these constraints could also act at the level of orthostichies, with the affected orthostichy contributing less to regrowth due to reduced photosynthetic capacity (Price and Hutchings 1992; Marquis 1996). Such limitations likely involve direct disruption of vascular integrity, e.g., impairing water and nutrient transport by creating non-functional xylem conduits and compromising phloem pathways (Brodersen and McElrone 2013). Additionally, herbivory reduces the photosynthetic capacity of wounded modules (Schultz et al. 2013). Together, these processes can significantly reduce the viability of heavily damaged tissues and impair the regrowth capacity of individual plants. In our study, herbivory levels were relatively low (20-30% leaf area removed for 2 to 4, out of 10-12 leaves per plant), compared to a previous study that found regrowth deficits in plants experiencing moderate to high mechanical damage (e.g., 50% of total leaf area removed in younger plants with 8–10 leaves; Quijano-Medina et al. 2019). This result suggests that concentrated damage can pose a significant challenge to regrowth in wild cotton, even at low to

291 moderate levels. Mechanisms such as resource reallocation (e.g., exchange of soluble sugars between
 292 leaves and roots; Quijano-Medina et al. 2019) and increased photosynthetic activity may not be
 293 sufficient to offset the negative impacts of herbivory under these conditions.

294 Consistent with previous work, EFN was more strongly induced locally, that is, when analyzing
 295 damaged leaves, and showed no or only weak systemic effects (Abdala-Roberts et al. 2019b; Briones-
 296 May et al. 2023). When comparing treatment groups, particularly regarding nectar sugar amounts, the
 297 high dispersed herbivory treatment induced the strongest EFN response and differed significantly from
 298 the control. While this finding contradicts our initial prediction (i.e., stronger resistance-related defense
 299 induction under concentrated damage), it still highlights the importance of herbivory variability—rather
 300 than total herbivory amount, at least within the levels tested—in shaping EFN responses. We
 301 hypothesize that signaling pathways associated with EFN induction may be more effectively activated
 302 when damage is spread across multiple modules, as risk signals may plateau within individual leaves
 303 but accumulate across damaged modules (de Bruxelles and Roberts 2001). At the same time, plants
 304 may limit EFN induction in heavily damaged modules to allocate resources toward other resistance-
 305 related traits, reflecting well-documented tradeoffs between defense strategies in plants (Moles et al.
 306 2013; Eichenberg et al. 2015). In turn, the observed EFN responses to within-plant variability in
 307 herbivore damage may have important downstream consequences for ant-plant interactions (Llandres et
 308 al. 2019; Vázquez-Barrios et al. 2021; Reyes-Hernández et al. 2022), which could explain EFN
 309 induction patterns. Supporting this, ongoing work shows that dispersed damage leads to a greater
 310 number of secreting nectaries (a response not measured here, presumably due to local induction), which
 311 increases ant recruitment and enhances herbivore attack rates (Ramírez-Delgado et al. under review).
 312 Thus, both increased EFN secretion per nectary (as shown here) and whole-plant level induction
 313 (perhaps more importantly) confirm greater EFN investment in response to dispersed damage,

314 mediating indirect defense via ant recruitment. These findings underscore that future studies should
 315 consider the effects of within-plant herbivory variability and its underlying mechanisms in upland
 316 cotton, as well as in other *Gossypium* species (e.g., see Wäckers et al. 2001; Rudgers et al. 2004) and
 317 other EFN-bearing myrmecophytic species.

318 The lack of a significant treatment effect on phenolic compounds levels was unexpected. Previous
 319 studies with wild cotton using artificial damage (e.g., mechanical wounding with *Spodoptera*
 320 *frugiperda* oral secretions or *S. frugiperda*-only damage) reported significant induction of phenolic
 321 compounds in preexisting leaves within 72 h and up to one week after damage onset (Abdala-Roberts
 322 et al. 2019b; Quijano-Medina et al. 2023). In addition, another study found significant induction of
 323 phenolic compounds one week after damage; however, these responses were generally weaker than
 324 those observed in newly developed leaves collected five weeks post-damage (Quijano-Medina et al.
 325 2021), suggesting that systemic phenolic responses in new tissues play a more important role in
 326 induced resistance. Earlier studies with cultivated cotton and wild cotton (Usha Rani and Pratyusha
 327 2013; Dixit et al. 2017), using generalist lepidopteran herbivores (*Spodoptera litura* and *Helicoverpa*
 328 *armigera*), also reported significant induction of phenolic compounds one to four days after damage
 329 onset, along with deleterious effects on herbivores. These findings suggest that phenolic compounds are
 330 generally highly inducible in cotton across a range of experimental approaches, herbivore species, and
 331 damage types (natural vs. artificial), and across both domesticated and wild cotton. However, we
 332 cannot rule out the possibility that peak induction occurred earlier than our sampling time (e.g.,
 333 between 48 and 72 h post-damage) or that systemic induction was stronger in newly developed leaves
 334 —an effect also observed for terpenoid aldehydes (Opitz et al. 2008; Eisinger et al. 2017, 2018.).
 335 Another possibility is that *A. argillacea*, as a specialist, suppresses phenolic compound production.
 336 Herbivore-mediated suppression of plant defenses is a known phenomenon (reviewed by Ali and

337 Agrawal 2012). It has been documented in both specialist systems, e.g., the suppression of
 338 glucosinolates in *Brassica oleracea* (Karssemeijer et al. 2022), and generalist systems, such as the
 339 suppression of green leaf volatiles in *Zea mays* (Jones et al. 2019), and jasmonic acid-mediated
 340 defenses in tomato by *Aculops lycopersici* (Glas et al. 2014), and *Arabidopsis thaliana* (Chen et al.
 341 2019). Consistent with our findings, ongoing work also shows non-significant induction by this insect
 342 across two ontogenetic stages of wild cotton, i.e., one month- vs four-month-old plants (Briones-May et
 343 al. under review). Further research is needed to test if *A. argillacea* is indeed able to suppress defenses
 344 in cotton, for instance by assessing defensive gene expression levels in response to varying (and
 345 potentially higher) caterpillar loads and comparing the responses with those induced by other
 346 caterpillar species.

347 **Gaps in research and directions**

348 Although our study provides novel insights into how herbivory patterns influence defense allocation,
 349 several questions remain. Future research should explore the temporal dynamics of these responses, as
 350 defense induction and tolerance mechanisms may operate on different timescales. Additionally,
 351 investigating whether these patterns hold across different herbivore species, particularly when
 352 comparing specialists to generalists, would further illuminate the context dependency of defense
 353 allocation strategies under varying herbivory scenarios. Examining the molecular mechanisms
 354 underlying differential responses to concentrated versus dispersed damage could also provide deeper
 355 insights into the signaling pathways that regulate resistance- and tolerance-related responses. Lastly, an
 356 important gap to address is the measurement of other types of chemical and structural defenses in
 357 cotton, such as terpenoid aldehydes and trichome density (pubescence).

358 **Conclusion**

359 Our study demonstrates that *G. hirsutum* tailors its defensive strategies based on the spatial dispersion
 360 of herbivory within individual plants, rather than simply responding to total damage levels. These
 361 results suggest that damage characteristics both enable adaptive responses under different threat
 362 scenarios and constrain defensive options. These findings highlight the importance of examining
 363 herbivory variability alongside mean damage levels when studying induced defenses. Understanding
 364 how plants have evolved to detect and respond to complex herbivory patterns carries broader ecological
 365 and evolutionary implications for plant-herbivore interactions.

366

367 **Data archiving**

368 The data and analysis scripts for this study have been deposited in the Zenodo repository and are
 369 available at [DOI]

370 **Conflict of interest**

371 The authors declare no conflict of interest.

372 **Funding**

373 This work was funded by a project awarded to TCJT (315230_185319) by the Swiss Science
 374 Foundation. VHR-D was supported by a postdoctoral fellowship from SECIHTI.

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Table 1. Statistical analysis of plant defense responses in relation to damage treatments and plant height. Results from general linear models for induced extrafloral nectar (EFN) volume and sugar production, and from linear mixed models for induced phenolic compounds and tolerance (dry weight of new leaves). Significant *p*-values (*p* < 0.05) are shown in bold. The table presents test statistics, degrees of freedom, and *p*-values for each effect (damage treatment or plant height), measured at two leaf levels when applicable.

Effect	Response variable						
	EFN volume		Total sugar		Total phenolics		Tolerance
	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2	
Damage treatment	$\chi^2 = 3.54$; df = 3; <i>p</i> = 0.3158	$\chi^2 = 32.69$; df = 3; <i>p</i> < 0.0001	$\chi^2 = 7.76$; df = 3; <i>p</i> = 0.0512	$\chi^2 = 21.84$; df = 3; <i>p</i> < 0.0001	F = 1.87; df = 3, 40.8; <i>p</i> = 0.1491	F = 0.56; df = 3, 40.08; <i>p</i> = 0.6427	F = 4.53; df = 3, 40.07; <i>p</i> = 0.0079
Plant height	$\chi^2 = 5.26$; df = 1; <i>p</i> = 0.0218	$\chi^2 = 5.77$; df = 1; <i>p</i> = 0.0163	$\chi^2 = 0.70$; df = 1; <i>p</i> = 0.4037	$\chi^2 = 4.19$; df = 1; <i>p</i> = 0.0227	F = 0.10; df = 1, 43.07; <i>p</i> = 0.7471	F = 0.24; df = 1, 44.78; <i>p</i> = 0.6257	F = 2.68; df = 1, 42.84; <i>p</i> = 0.1085

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Figure captions

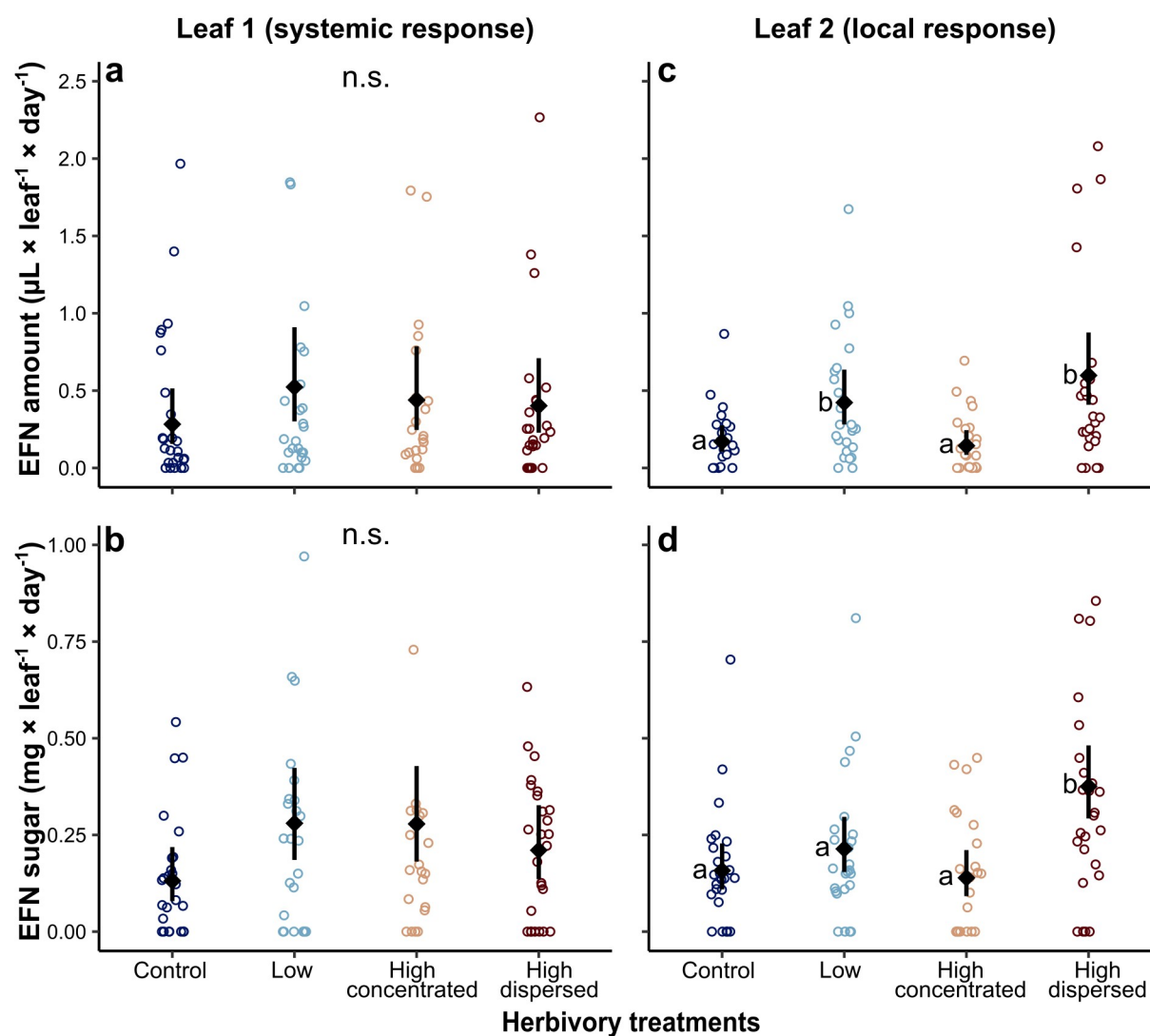
Fig. 1 Effects of damage amount and dispersion on extrafloral nectar (EFN) production, in terms of nectar volume and total sugar content, in wild cotton (*Gossypium hirsutum*). Panels show: (a) leaf 1 (undamaged, reflecting systemic induction) and (b) leaf 2 (damaged, reflecting local induction) for EFN volume (μl) produced and total sugars (mg) in response to damage treatments. Colored circles represent individual plant values for each herbivory treatment (dark blue: control, blue: low, yellow: high concentrated, dark red: high dispersed herbivore). Black squares and error bars indicate estimated marginal means and 95% confidence intervals from general linear mixed models. Different letters indicate significant differences among treatments (Tukey post-hoc tests, $P < 0.05$); “n.s.” denotes non-significant differences.

Fig. 2 Effects of damage amount and dispersion on phenolics compound production in wild cotton (*Gossypium hirsutum*). Panels show: (a) leaf 1 (systemic response) and (b) leaf 2 (local response), with phenolic concentrations expressed as tannic acid equivalents (TAE) per gram of leaf dry weight (DW). Colored circles represent individual plant values for each herbivory treatment (dark blue: control, blue: low, yellow: high concentrated, dark red: high dispersed herbivore). Black squares and error bars indicate estimated marginal means and 95% confidence intervals from linear mixed models. There were no significant differences among treatments, as indicated by “n.s.”

Fig. 3 Effects of herbivory amount and within-plant variability on the biomass of newly developed leaves, measured as grams of dry weight (DW) after the herbivory treatments were applied. Colored circles represent individual plant values for each treatment (dark blue: control, blue: low, yellow: high concentrated, dark red: high dispersed herbivore). Black squares and error bars indicate estimated marginal mean and 95% confidence intervals from linear mixed models. Different letters indicate significant differences among treatments (Tukey post-hoc test, $P < 0.01$).

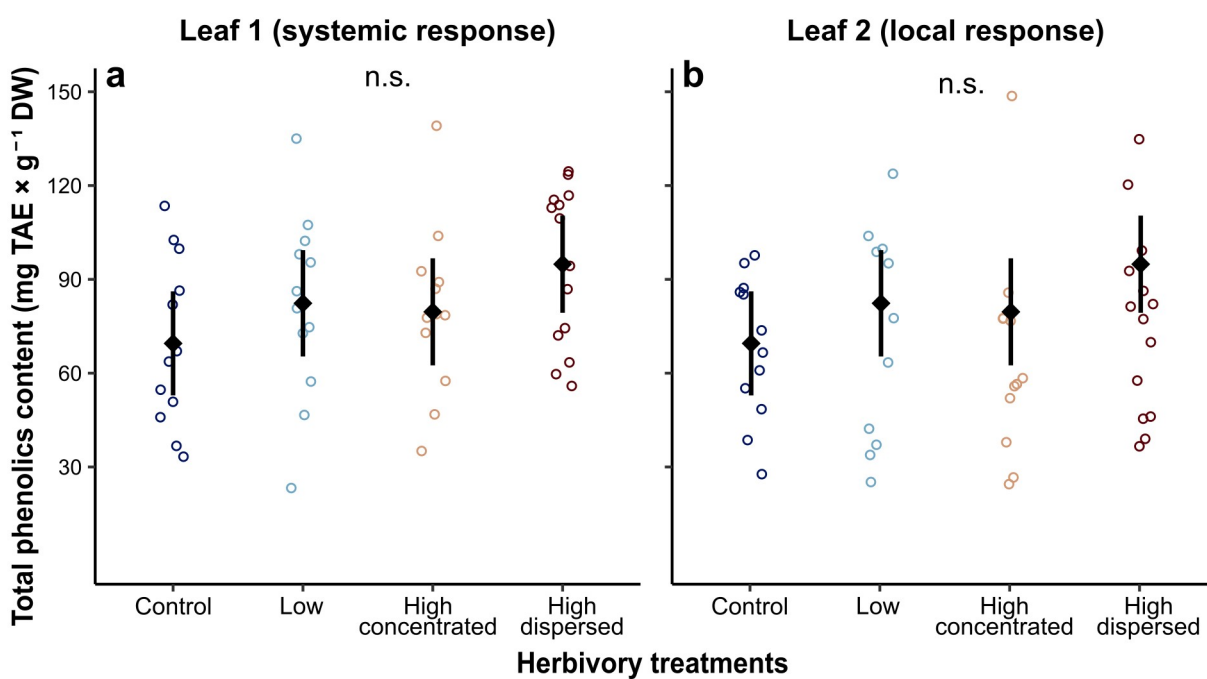
Figures

Fig. 1



628 **Fig. 2**

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630 **Fig. 3**

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