


RESEARCH PAPER

Compensation to simulated insect leaf herbivory in wild cotton (*Gossypium hirsutum*): responses to multiple levels of damage and associated traits

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Editor

A. Pineda

Received: 21 January 2019; Accepted: 29 April 2019

doi:10.1111/plb.13002

ABSTRACT

- Identifying the mechanisms of compensation to insect herbivory remains a major challenge in plant biology and evolutionary ecology. Most previous studies have addressed plant compensatory responses to one or two levels of insect herbivory, and the underlying traits mediating such responses remain elusive in many cases.
- We evaluated responses associated with compensation to multiple intensities of leaf damage (0% control, 10%, 25%, 50%, 75% of leaf area removed) by means of mechanical removal of foliar tissue and application of a caterpillar (*Spodoptera exigua*) oral secretions in 3-month-old wild cotton plants (*Gossypium hirsutum*). Four weeks post-treatment, we measured plant growth and multiple traits associated with compensation, namely: changes in above- and belowground, biomass and the concentration of nutrients (nitrogen and phosphorus) and non-structural carbon reserves (starch and soluble sugars) in roots, stems and leaves.
- We found that wild cotton fully compensated in terms of growth and biomass allocation when leaf damage was low (10%), whereas moderate (25%) to high leaf damage in some cases led to under-compensation. Nonetheless, high levels of leaf removal (50% and 75%) in most cases did not cause further reductions in height and allocation to leaf and stem biomass relative to low and moderate damage. There were significant positive effects of leaf damage on P concentration in leaves and stems, but not roots, as well as a negative effect on soluble sugars in roots.
- These results indicate that wild cotton fully compensated for a low level of leaf damage but under-compensated under moderate to high leaf damage, but can nonetheless sustain growth despite increasing losses to herbivory. Such responses were possibly mediated by a re-allocation of carbohydrate reserves from roots to shoots.

INTRODUCTION

Plants have evolved a variety of induced responses against herbivory (Fineblum & Rausher 1995; Strauss & Agrawal 1999; Núñez-Farfán *et al.* 2007). On the one hand, following herbivore attack, plants can deter, delay or reduce the amount of herbivore damage by mechanisms of induced resistance, such as increased production of chemical and physical defensive traits (Núñez-Farfán *et al.* 2007; Agrawal 2011; Karban 2011). On the other hand, plants can mitigate the negative effects of herbivory through compensatory mechanisms, such as increased storage capacity, increased photosynthesis rates, compensatory growth and reproduction, and changes in nutrient and carbon allocation or uptake (Strauss & Agrawal 1999; Stowe *et al.* 2000; Tiffin 2000; Fornoni 2011). Although these modes of plant defence play an important role in reducing the negative impacts of herbivory, compensatory responses and their underlying mechanisms are comparatively less understood than those associated with induced resistance, but are nonetheless arguably widespread (Garcia and Eubanks 2019; Ramula *et al.* 2019).

A common limitation in previous studies has been that the plant traits mediating compensatory responses to herbivory are frequently overlooked (reviewed by Strauss & Agrawal 1999; Stowe *et al.* 2000; Tiffin 2000; Fornoni 2011). The relatively few studies which have addressed these underlying defence traits have found that compensation in growth and reproduction is frequently linked with greater investment in underground tissues to enhance nutrient uptake (*e.g.* Hochwender *et al.* 2000; Stevens *et al.* 2008), as well as with the re-allocation of carbon reserves for tissue regrowth (*e.g.* Rivera-Solís *et al.* 2012) and with increased leaf photosynthesis rates (Stevens *et al.* 2008). Other studies have found that nutrients (*e.g.* nitrogen and phosphorus) and non-structural carbohydrates (*e.g.* starch and sugars; Gómez *et al.* 2010; Moreira *et al.* 2012) are diverted away from the site of damage and into storage tissues, leading to reduced nutrient and carbon concentration in attacked plant tissues (Newingham *et al.* 2007; Gómez *et al.* 2010). These short-term gains in resource storage in turn mediate compensation to subsequent herbivory. Together, results therefore emphasise the importance of simultaneously assessing multiple plant traits associated with compensatory ability

and underlying processes (e.g. resource re-allocation patterns) in order to more robustly understand plant compensation to herbivory (Fornoni 2011).

A plant's capacity to compensate for herbivory depends on the amount or severity of herbivore damage (Tiffin 2000; Fornoni 2011; Ramula *et al.* 2019). Nevertheless, studies usually test one or a few levels of damage and this has resulted in a limited understanding of plant tolerance, including features such as thresholds in compensatory responses to varying intensities of herbivore damage (Núñez-Farfán *et al.* 2007; Fornoni 2011; Poveda *et al.* 2018). For example, a study by Huhta *et al.* (2000) found that field gentian (*Gentianella campestris*) plants compensated in terms of growth rate up to damage levels of 50% of leaf area removed, but exhibited marked under-compensation with 75% leaf damage. In contrast, Blue *et al.* (2015) found that lima bean (*Phaseolus lunatus*) plants under-compensated to a similar degree in terms of both above- and belowground biomass production when subjected to 33 or 66% leaf area removal. These two studies highlight the need to account for multiple levels (or even a continuum) of damage to better characterise the function (*i.e.* shape) and thresholds of plant compensatory responses to herbivory (e.g. level at which compensation is compromised).

In this study, we investigated whether varying levels of simulated herbivory by leaf-chewing insects differentially affected growth and a number of traits putatively associated with plant compensation to herbivory. To this end, we performed a greenhouse experiment with 3-month-old saplings of wild cotton, *Gossypium hirsutum* (*Malvaceae*), subjected to multiple levels of mechanical damage (0%, 10%, 25%, 50% and 75% leaf area removal) combined with the application of caterpillar (*Spodoptera exigua*, Lepidoptera) oral secretions. We measured plant growth as well as traits (e.g. concentration of nutrients and non-structural carbon reserves) potentially associated with compensatory growth or changes in biomass allocation among plant tissues following leaf herbivory. We hypothesised full compensatory responses in cotton plants under lower levels of damage due to the use of reserves in the different tissues, whereas increasing damage would potentially compromise compensation mechanisms. Overall, the present work builds towards a better understanding of plant responses associated with compensation to varying intensities of herbivory and their underlying mechanisms.

MATERIAL AND METHODS

Natural history

Gossypium hirsutum (*Malvaceae*) is a perennial shrub which grows up to 2-m tall (Oosterhuis & Jernstedt 1999). It is native to Central America, Mexico and the Caribbean Basin (Wendel *et al.* 1992; Oosterhuis & Jernstedt 1999) and is thought to have originated in southeast Mexico where it was initially domesticated (D'Eeckenbrugge & Lacape 2014). Wild populations of this species are particularly common along the northern coast of the Yucatan Peninsula (Mexico), growing in the coastal scrubland and sand dune vegetation (D'Eeckenbrugge & Lacape 2014). Along its native range in the Yucatan Peninsula, wild cotton is attacked by a diverse community of insect herbivores, among which leaf chewers (e.g. Orthoptera, and larval

stages of native Lepidoptera and Coleoptera) are especially common (L. Abdala-Roberts, unpublished data).

Plant material and experimental design

In May 2017, we collected seeds from 18 plants (*i.e.* maternal lines) of wild cotton from a naturally occurring population located on the northeast coast of the Yucatan Peninsula (Quintana Roo, Mexico; 20°45'36.6" N, 86°58'12.9" W). Previous genetic work based on samples taken at nearby sites indicated that populations in this area contain mixtures of wild and feral genotypes (see D'Eeckenbrugge & Lacape 2014). In August 2017, we grew all plants individually from seeds sowed in 4-l pots containing potting soil with peat. Throughout the duration of the experiment, plants were grown in a glasshouse under controlled conditions (12 h light per day, 10 °C night, 25 °C day) at the Mision Biologica de Galicia (Spain), and were watered twice a week.

Three months after seed germination, we selected 100 plants for which we measured total height and counted the number of fully expanded leaves. We then randomly assigned 20 plants to one of five treatments: (i) undamaged control, (ii) 10% of leaf area removed, (iii) 25% of leaf area removed, (iv) 50% of leaf area removed and (v) 75% of leaf area removed. Previous surveys indicated that the population mean level of leaf damage by insects on wild cotton is $22.49 \pm 2.12\%$ (mean \pm SE), range: $9.22 \pm 0.87\%$ to $52.13 \pm 8.06\%$ across populations ($N = 26$), whereas leaf damage at the individual plant level ranges from 3% to 88% of leaf removal (L. Abdala-Roberts, unpublished data). Therefore, herbivory treatment levels fell within the natural range of herbivory by leaf-chewing insects observed for natural populations of this species. In particular, treatments of 10% and 25% leaf removal evaluated compensatory responses to low and moderate amounts of leaf damage, respectively, whereas 50% and 75% leaf removal evaluated responses to high levels of leaf damage by chewing insects. Treatment application involved the removal of the desired level of damage from the sides of each leaf using scissors, as well as puncturing the remaining central portion of the leaf blade with an awl (4–5 holes leaf⁻¹). Immediately after, we exposed the punctured portion of the leaf to oral secretions from third instar larvae of the beet armyworm (*Spodoptera exigua*) by gently poking the abdomen of each caterpillar (see Turlings *et al.* 1993). We used two to three caterpillars per plant, depending on the number of leaves. Although applying mechanical damage plus insect oral secretions is less realistic compared to natural insect damage, previous studies have shown that this approach provides an effective proxy for natural damage in several cultivated species (for maize see Turlings *et al.* 1993 and Alborn *et al.* 1997; for tobacco see Halitschke *et al.* 2001), including wild cotton (Chappuis & Egger 2016; Abdala-Roberts *et al.* 2019). This approach allowed us to elicit realistic levels of plant defence induction while precisely controlling the amount of leaf tissue removed, and therefore provided a robust evaluation of plant compensatory responses to herbivory by chewing insects on wild cotton. Prior to application of oral secretions, we reared larvae of *S. exigua* on a wheatgerm-based artificial diet. For most maternal lines we lacked plant replication for two or more of the damage treatment levels and were therefore not able to test for plant genotypic variation in the treatment effect.

Our leaf damage treatment focused exclusively on the effects of chewing insects, as this guild is by far the most common type of attacker (e.g. relative to leaf miners and phloem feeders) in natural populations of wild cotton (Abdala-Roberts *et al.* 2019), and therefore represented the most important source of insect leaf herbivory. In addition, wild cotton can experience relatively high levels of damage during short periods of time (1–2 days) due to insect outbreaks (e.g. locusts and, to a lesser extent, caterpillars; T. Quijano-Medina personal observation), such that the application of single events of varying intensity of leaf damage do in fact represent a possible scenario of herbivore attack under field conditions. It is important to also note that although *S. exigua* is not a common herbivore on wild cotton throughout this plant's native range, it is one of the most important pests on cultivated cotton (Brown & Dewhurst 1975; Capinera 2001) and has been used in a number of previous studies to assess direct and indirect induced resistance in cultivated varieties of *Gossypium* spp. (e.g. Loughrin *et al.* 1995; Bezemer *et al.* 2004). This insect was therefore considered a suitable study model for assessing compensatory responses to herbivory by a generalist insect in wild *G. hirsutum*.

Sampling and response variables measured

Four weeks post-treatment, we measured plant height, counted the number of new leaves, harvested all plants and transported the plants in ice coolers to the laboratory where we separated leaves, stems and roots. Plant material was oven-dried for 72 h at 65 °C to constant weight and weighed to the nearest 0.0001 g. We only measured biomass for new leaves, since leaf area removal due to treatment application would influence measurements of total leaf biomass. For each plant tissue (new leaves, stems and roots), we also measured the concentration of nutrients (N and P) for half of the plants per treatment level, as well as starch and soluble sugars (non-structural carbohydrate reserves) for the other half of the plants.

Plant height and the production of new leaves are proxies for growth and are commonly used to measure compensatory growth, a broadly accepted compensatory mechanism against herbivory (Strauss & Agrawal 1999; Fornoni 2011; Robert *et al.* 2014; Moreira *et al.* 2015). In addition, plant compensation to aboveground herbivory frequently involves increased biomass allocation to below- relative to aboveground tissues in order to improve root water and nutrient uptake, which in turn mediates aboveground regrowth (Erb *et al.* 2009; Moreira *et al.* 2012). Relatedly, changes in N and P levels frequently result from increased root nutrient uptake, within-plant nutrient re-allocation to new tissues, as well as increased photosynthesis rates in response to damage (Babst *et al.* 2005; Gómez *et al.* 2010; Moreira *et al.* 2012). Moreover, plant mobilisation of stored (non-structural) carbon reserves from belowground to aboveground tissue upon foliar damage is also a common mechanism of regrowth following herbivore damage (Tiffin 2000; Rivera-Solís *et al.* 2012; Piper & Fajardo 2014; Robert *et al.* 2014). A period of 4 weeks after defoliation was chosen to measure changes in plant growth and in plant traits associated with compensation to herbivory in wild cotton since individuals start producing new leaves approximately 10–14 days after defoliation (L. Abdala-Roberts, personal observation). Accordingly,

production of new leaves as well as stem elongation and leaf expansion occurring over the following weeks post-treatment would presumably be mediated by compensatory mechanisms, such as changes in resource uptake, biomass allocation, photosynthesis rates and resource re-allocation processes affecting nutrients and non-structural carbon reserve levels.

Chemical analyses

To quantify N and P concentration in leaves, stems and roots, we selected ten plants per treatment and digested approximately 0.1 g ground dried leaf material in a mixture of selenous (H_2SeO_3) sulphuric acid and hydrogen peroxide (Moreira *et al.* 2012). We then used a colorimetric analysis of diluted aliquots of the digested sample to quantify N (indophenol blue method) and P (molybdenum blue method) concentration using a Bio-Rad 650 microplate reader (Bio-Rad Laboratories, Philadelphia, PA, USA) at 650 and 700 nm, respectively (Walinga *et al.* 1995). We expressed N and P concentration in $\text{mg}\cdot\text{g}^{-1}$ tissue on a dry weight basis.

To quantify the concentration of soluble sugars and starch in leaves, stems and roots, we selected ten plants per treatment (only four for new leaves due to the low amount of plant material) and used the anthrone method (Hansen & Møller 1975). Briefly, we extracted soluble sugars from finely ground leaves (50 mg) with aqueous ethanol (80% v/v). We extracted starch with 1.1% hydrochloric acid in a water bath at 100 °C for 30 min, followed by centrifugation and subsequent dilution of the extract (Sampedro *et al.* 2011). We determined soluble sugars and starch concentration colorimetrically in a Bio-Rad 650 microplate reader at 630 nm, using glucose and potato starch, respectively, as standards. Soluble sugar and starch concentrations were also expressed in $\text{mg}\cdot\text{g}^{-1}$ tissue on a dry weight basis.

Statistical analyses

First, we performed general linear mixed models (GLMM) to test for an effect of leaf damage (fixed, five levels) on growth-related variables, *i.e.* plant height and number of new leaves. The model for plant height also included initial height as a covariate to control for initial differences in size, whereas the model for new leaf number included the number of leaves prior to treatment application to control for pre-treatment differences in leaf number potentially influencing re-growth. Second, we ran GLMMs testing for an effect of leaf damage on biomass, as well as nutrient (N and P) and non-structural carbohydrate (starch and soluble sugar) concentrations. In each case, models were performed separately for stems, roots and new leaves. In addition, we also ran a model testing for effects of leaf damage on the ratio of shoot:root biomass (where shoot biomass = stem + leaf biomass).

All of the models described above included plant genotype as a random effect to control for variation among maternal lines. We did not test for the significance of this effect as it was not among the goals of this study, and even if we had tested this it would have been of limited use due to the strong imbalance in sample sizes among maternal lines. We log-transformed biomass variables to achieve normality of residuals and report model least-square means \pm SE (back-transformed for

transformed data) as descriptive statistics. If the treatment effect was significant, we ran subsequent pair-wise comparisons among treatment level means and reported Bonferroni-corrected *P*-values. Analyses were performed using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Plant growth and biomass

We found a significant treatment effect on plant height (Fig. 1A): treatments of 25%, 50% and 75% leaf area removal significantly reduced plant height compared to control and 10% leaf removal (Fig. 1A). Control and 10% leaf removal did not differ significantly (Fig. 1A), indicating that plants fully compensated for low leaf damage. In addition, we found no significant treatment effect on the number of new leaves, indicating full compensation in all cases (Fig. 1B).

Biomass responses for new leaves was significantly different in relation to those for stems and roots (Fig. 1C–E): treatments of 25%, 50% and 75% leaf removal reduced the biomass of new leaves compared to that of controls (Fig. 1C). Treatments of 25% and 50% leaf removal did not differ from 10% leaf removal, whereas controls and 10% leaf removal were similar (Fig. 1C). On the other hand, treatments of 25% and 75% leaf removal reduced stem biomass compared to controls (Fig. 1D). The treatment of 25% leaf removal did not differ from 10% and 50% leaf removal in terms of stem biomass, whereas control, 10% and 50% leaf removal were similar (Fig. 1D). Finally, we found that 75% leaf removal reduced root biomass compared to controls and all other treatment levels (Fig. 1E).

Control and 10%, 25% and 50% leaf area removed treatments did not differ significantly in terms of root biomass (Fig. 1E). There was no significant treatment effect on shoot:root biomass ratio ($F_{4,83} = 0.55$, $P = 0.69$).

Nutrient concentrations

We found a significant treatment effect on the concentration of P in new leaves and stems (Fig. 2A and B), but not in roots (Fig. 2C). All levels of leaf damage increased the concentration of P in new leaves compared to controls (Fig. 2A). Leaf damage levels of 25% and more (but not 10%) increased the concentration of P in stems compared to controls, and 25% leaf removal had a significantly larger mean value than 50% and 75% damage (Fig. 2B). There was no significant treatment effect on leaf N concentration for any plant part (Fig. 2D–F).

Non-structural carbon reserves

We found a significant treatment effect on the concentration of soluble sugars in roots (Fig. 3C), but not in leaves or stems (Fig. 3A and B). Treatment of 75% leaf removal significantly reduced the concentration of soluble sugars in roots compared to control, 10% and 25% leaf area removal, but did not differ from 50% leaf removal (Fig. 3C). The 50% leaf removal treatment did not differ from controls but significantly reduced soluble sugars compared to 10% and 25% leaf removal (Fig. 3C). The 10% and 25% leaf removal treatments did not differ from controls or between treatments (Fig. 3C). There were no significant effects of leaf damage treatment on starch concentration for any plant part (Fig. 3D–F).

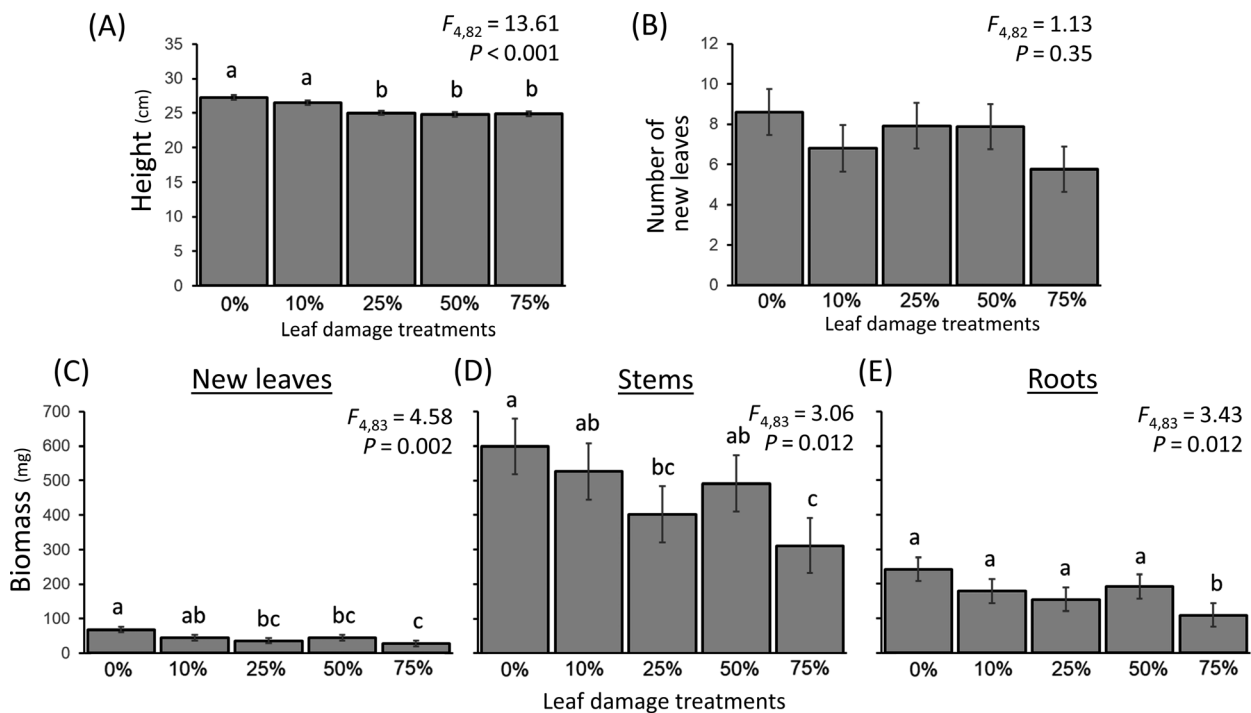


Fig. 1. Effect of leaf damage treatments (0% undamaged control, 10% of leaf area removed, 25% leaf area removed, 50% leaf area removed, 75% leaf area removed) on (A) height (cm), (B) number of new leaves and biomass (mg dry weight) of (C) new leaves, (D) stems and (E) roots of wild *Gossypium hirsutum* plants. Bars are least-square means \pm SE ($N = 20$ plants treatment $^{-1}$). Different letters indicate significant ($P < 0.05$) differences between treatment levels.

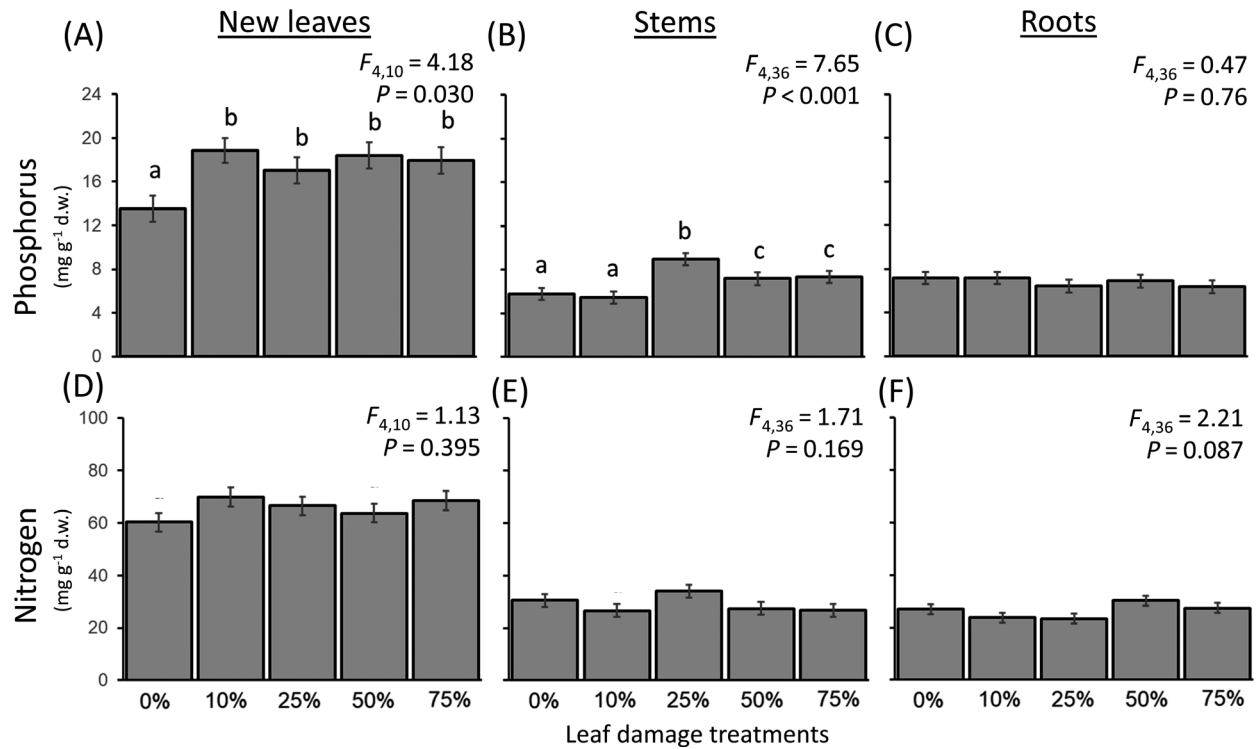


Fig. 2. Effect of leaf damage treatments (0% undamaged control, 10% leaf area removed, 25% leaf area removed, 50% leaf area removed, 75% leaf area removed) on the concentration of phosphorus and nitrogen (mg g⁻¹ dry weight) in new leaves (A, D), stems (B, E) and roots (C, F) of wild *Gossypium hirsutum* plants. Bars are least-square means \pm SE (N = 10 plants per treatment). Different letters indicate significant ($P < 0.05$) differences between treatment levels.

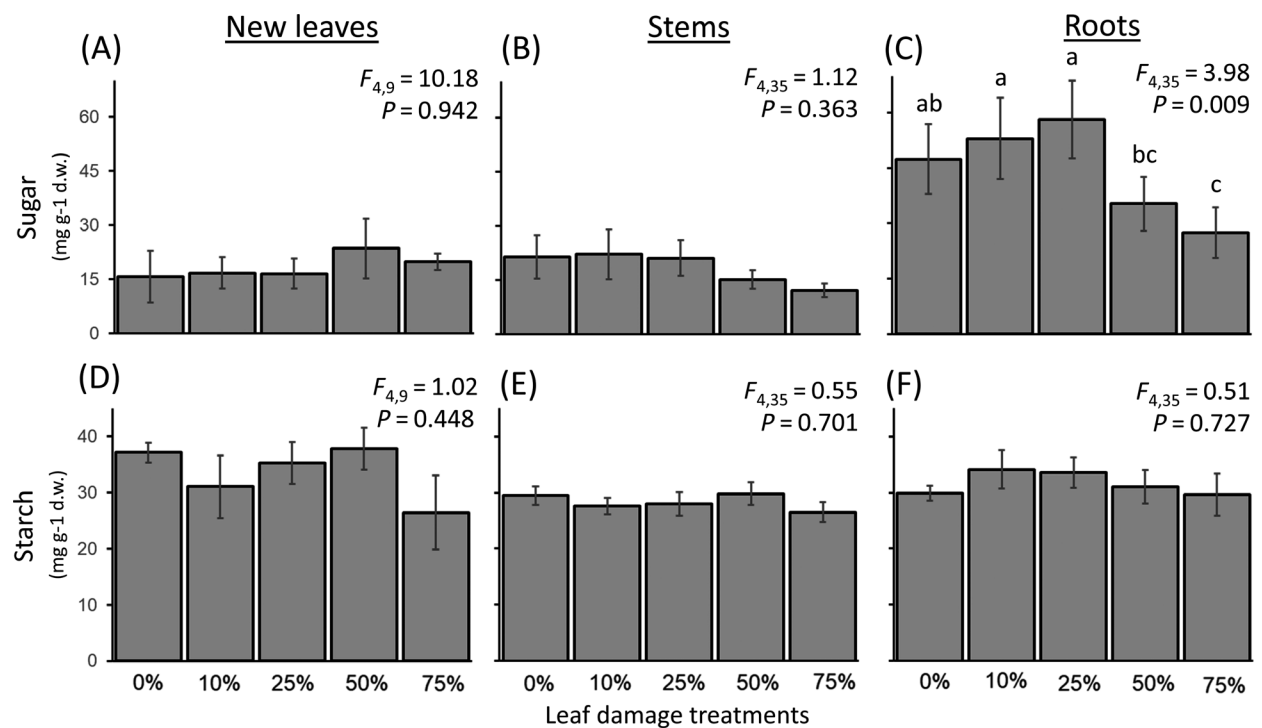


Fig. 3. Effect of leaf damage treatments (0% undamaged control, 10% leaf area removed, 25% leaf area removed, 50% leaf area removed, 75% leaf area removed) on the concentration of sugars and starch (mg g⁻¹ dry weight) in new leaves (A, D), stems (B, E) and roots (C, F) of wild *Gossypium hirsutum* plants. Bars are least-square means \pm SE (N = 10 plants per treatment for stems and roots, N = 4 plants per treatment for new leaves). Different letters indicate significant ($P < 0.05$) differences between treatments levels.

DISCUSSION

Synthesis

Our findings indicated that 3-month-old saplings of wild cotton fully compensated for low leaf herbivory (10% leaf removed). However, when challenged to moderate or high levels of leaf damage, plant responses ranged from under-compensation to full compensation, depending on the response variable and level of damage. Cotton fully compensated for moderate leaf damage (25%) in terms of root biomass but not height, stem or leaf biomass. At high damage levels, full compensation to 50% leaf removal was observed for stem and root biomass but not plant height or leaf biomass, whereas at 75% damage plants consistently under-compensated across most variables measured. Interestingly, however, treatment levels of moderate to high leaf removal in several cases did not differ (e.g. height, leaf biomass), indicating that increasing levels of herbivory did not lead to concomitant further reductions in cotton compensation. Together, these results suggest that wild cotton plants are able to fully compensate for low and even moderate leaf damage, as well as sustain compensatory responses despite increasing losses to herbivory. Finally, changes in plant growth and biomass were associated with concomitant changes in plant traits, with damaged plants exhibiting a significantly higher concentration of leaf P and lower concentration of soluble sugars in roots. These trait changes were potentially associated with biochemical and physiological mechanisms underlying wild cotton compensation to leaf damage.

Effects of leaf damage treatments on plant growth and biomass allocation

The results suggest that wild cotton plants were able to fully compensate in terms of growth (measured as height) and biomass allocation when damage was low (10% leaf area removed), whereas 25% or more leaf area removed led to under-compensation. The fact that 25% leaf removal led to reductions in growth, and this level of damage is close to the mean value observed under field conditions, suggests that under-compensation is relatively common in wild cotton populations. At the same time, however, two lines of evidence suggest that cotton compensatory responses to herbivory are biologically important. First, the reductions (relative to controls) in plant height and biomass of new leaves under 25%, and in some instances also 50% leaf damage, albeit significant, were not substantial, suggesting that moderate to high levels of herbivory do not lead to biologically large reductions in plant growth or biomass allocation. Second, 25% and 50% leaf damage in many cases did not differ from 10% damage, indicating that plants were able to sustain growth despite increasing damage, suggesting that compensatory responses are able to maintain plant growth and biomass allocation despite increasing losses to herbivory. Only when leaf damage was highest (75%) did wild cotton plants consistently exhibit under-compensation; however, this intensity of herbivory is uncommon for most of the populations sampled thus far (L. Abdala-Roberts, unpublished data).

Previous work with herbaceous and woody plants (including *Gossypium hirsutum*) have similarly found full compensation and sustained growth up to moderate levels of damage (e.g. 20–40%), whereas high levels of damage (e.g. >40%) frequently compromise plant growth and reproductive output (Kerby &

Keeley 1987; Rosenheim *et al.* 1997; Huhta *et al.* 2000; Del-Val & Crawley 2005; Akiyama & Ågren 2012). That said, full compensation or over-compensation has been reported from low to high levels of severity of herbivore damage for a number of plant species (reviewed by Ramula *et al.* 2019). A recent meta-analysis also indicated that reproductive compensation to herbivory tended to be stronger, on average, for woody than for herbaceous plants (Garcia and Eubanks 2019), suggesting the latter have evolved mechanisms for greater tolerance to herbivory. Thus, the results suggest that wild *G. hirsutum* could have similar compensatory capacity relative to other herbaceous plants previously studied, and overall falls at an intermediate point along the spectrum of compensatory responses for both herbaceous and woody species.

Effects of leaf damage on nutrient and carbon reserves

The effects of leaf damage on nutrient and carbon reserves revealed distinct patterns likely associated to compensatory responses in growth and biomass allocation. For example, the observed increase in leaf P concentration in damaged plants could be explained by the resorption of this nutrient from senescent tissues and damaged leaves, and its re-allocation to new (undamaged) photosynthetic tissues (Moreira *et al.* 2012; Veneklaas *et al.* 2012). A similar, albeit non-significant, trend was also observed for leaf N concentration in damaged plants. This accumulation of P suggests a re-adjustment of biochemical processes associated with compensatory responses in above-ground tissues, such as increased photosynthetic activity in new leaves (Meyer 1998; Strauss & Agrawal 1999), and could have mediated observed aboveground growth responses in wild cotton. It is also possible, however, that P levels increased in undamaged leaves due to loss of leaf tissue (*i.e.* ‘concentration effects’; e.g. Koricheva 1999; Moreira *et al.* 2012) and not to metabolic changes mediating compensatory responses. It should also be noted that short-term responses to defoliation (e.g. within a few days post-damage) could have also influenced nutrient levels, but would have been overlooked in our study as we quantified nutrients 4 weeks post-treatment. Further work assessing changes in nutrient levels at multiple time points (including short-term responses), as well as additional measurements to determine patterns of nutrient use and acquisition is necessary to obtain a better understanding of whether changes in nutrient levels following leaf damage mediate compensation in wild cotton.

Changes in the concentration of soluble sugars in roots due to leaf damage were also indicative of plant biochemical changes and physiological re-adjustment. The observed decrease in soluble sugars in belowground tissues subjected to the highest level of leaf damage (75%) could have reflected a reduction in supply of photosynthate from aboveground tissues to roots due to resource re-allocation from below- to aboveground tissues or changes in photosynthesis levels in aboveground tissues. These resource allocation changes among plant tissues are a common mechanism for compensation. For example, increased allocation of nutrients and photosynthate to aboveground tissues and reduced allocation to belowground tissues have been reported for several herbaceous species, particularly during early stages of plant development when carbon reserves are low or absent (Zhang & Romo 1994; Meyer 1998; Moreira *et al.* 2012). In our study, we found no treatment

effect on starch concentrations, suggesting that wild cotton plants do not make use of carbon reserves, as this would have resulted in a depletion of starch in roots and a concomitant increase in soluble sugars in shoots. In contrast, other studies have found different patterns of allocation of carbon reserves in response to herbivory in herbaceous species. Rivera-Solís *et al.* (2012) reported that leaf damage decreased starch concentrations in roots of *Ruellia nudiflora*, a perennial herb that is characterised by strong allocation to root growth and carbon reserves starting at early life stages (see also Babst *et al.* 2005; Schwachtje *et al.* 2006). Such a reduction in starch levels presumably contributed to full compensation to (40%) leaf area removal in this plant. In addition, a previous study with maize seedlings reported patterns of re-allocation of carbohydrates from damaged to undamaged stems after herbivory in roots, which presumably mediated re-growth of stem-borne roots and increased stem biomass (Robert *et al.* 2014). In our study, however, the role of carbon reserves appears to be less important in young wild cotton saplings, where plants apparently sacrifice root growth in the short term (cutting sugar supply to roots) in order to sustain aboveground growth, rather than making use of reserves to mediate simultaneous investment in above- and belowground tissues. Alternatively, cotton plants may use other forms of carbon storage in roots (e.g. oligosaccharides), which were mobilised to stems and leaves to mediate aboveground compensatory responses. Finally, we cannot discard the possibility that reductions in soluble sugars in roots post-treatment resulted simply from a loss of source (*i.e.* leaf) tissue, limiting sugar production and thus allocation from above- to belowground tissues. Whatever the mechanism, we should keep in mind that repeated events of herbivory could compromise belowground (and aboveground) growth; reductions in investment in root growth might be sustainable in the short term to mediate aboveground responses (as observed here), but repeated attacks would potentially further reduce root growth and nutrient uptake and ultimately constrain aboveground regrowth (Zhang & Romo 1994; Del-Val & Crawley 2005).

When interpreting the above findings, it is important to point out that a robust connection between changes in plant growth and leaf traits following leaf damage cannot yet be made based on our current findings. Further work with additional design features and measurement is necessary before stronger conclusions can be reached on compensatory mechanisms in wild cotton. First, an experimental set-up using plant genotypes (e.g. half- or full-sib lines) is desirable to accurately measure compensatory ability and relate it to candidate plant traits and underlying mechanisms (Núñez-Farfán *et al.* 2007; Fornoni 2011). Second, longer-term measurements of growth (and even reproduction) would allow us to more robustly relate compensation to plant fitness measures and, in this way, address the evolutionary implications of plant compensatory responses to tolerance to herbivory (Fornoni 2011). Further work addressing these features is currently underway and will allow more robust testing for compensatory responses in wild cotton.

Conclusions and future analysis

Overall, our findings suggest that young wild cotton plants are able to fully compensate for low levels of leaf damage by leaf-chewing insects, but that compensation might be

compromised at moderate to high levels of herbivory. This species appears to rely little on carbon reserves to mediate such responses during early plant growth, and instead achieves aboveground compensation possibly through a reduction in allocation of resources to belowground tissues. We therefore describe three lines of inquiry which, in our view, would be valuable to address in future work. First, investigate ontogenetic variation in cotton compensatory responses by including both early and later stages of plant development, which should reveal ontogenetic trajectories in plant compensation to herbivory (Del-Val & Crawley 2005). Associating compensatory responses with plant fitness components in early (e.g. survival) or later (e.g. fruit or seed output) stages of development would be important within this context. Second, previous work has suggested that tolerance to abiotic (e.g. water limitation or high temperatures) and biotic (e.g. herbivory, pathogen infection) stresses are not independent (Huhta *et al.* 2000; Pearse *et al.* 2017), as both types of response are in many cases mediated by the same resource use and acquisition traits. Future work focusing on the effects of abiotic variables on compensation to herbivory in wild cotton will contribute to address this. Alternatively, high tolerance to abiotic stress might, in some cases, involve a reduction in plasticity due to re-routing of traits that mediate abiotic resistance (Pearse *et al.* 2017), which could limit compensation to herbivory or pathogens. In either case, mechanisms of abiotic and biotic tolerance may interact and therefore merit joint consideration. Experiments factorially testing for the independent and interactive effects of biotic and abiotic stress on traits associated with compensation would be essential to this end, while also including realistic manipulations of abiotic factors based on conditions naturally experienced by plants *in situ*. This consideration could be particularly relevant for wild cotton plants, which are restricted to distinctly hostile environments characterised by elevated temperatures, low precipitation and high salinity. Third, addressing the influence of repeated or recurrent herbivory on compensatory responses is important, as repeated bouts of herbivory have been shown to compromise the compensatory ability of cultivated cotton plants (Kerby & Keeley 1987). Doing so would allow more full characterisation of the limits to compensation in this species in response to variation in both the severity and recurrence of herbivore attack (Underwood 2012). In all of the above scenarios, the identification of plant traits mediating dual functions (in response to biotic and abiotic stresses) and their degree of plasticity will provide a more robust understanding of plant compensatory mechanisms.

ACKNOWLEDGEMENTS

We thank María Sol Campañó, Andrea Galmán and María Dolores Pintos for help with greenhouse and laboratory tasks. Comments and suggestion by the subject editor (Ana Pineda), Kasey Barton and an anonymous reviewer helped to improve the manuscript. This research was financially supported by a Spanish National Research Grant (AGL2015-70748-R), a Regional Government of Galicia Grant (IN607D 2016/001) and the Ramón y Cajal Research Programme (RYC-2013-13230) to XM and a grant from the i-COOP+ CSIC Program (COOPB20158) to XM and LAR.

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