Herbivory-induced jasmonates constrain plant sugar accumulation and growth by antagonizing gibberellin signaling and not by promoting secondary metabolite production

Ricardo A. R. Machado[1,2,3], Ian T. Baldwin[2] and Matthias Erb[1,2,3]

[1]Root–Herbivore Interactions Group, Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany; [2]Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany; [3]Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

Authors for correspondence:
Ricardo A. R. Machado
Tel: +41 31 631 8814
Email: ricardo.machado@ips.unibe.ch

Matthias Erb
Tel: +41 31 631 8668
Email: matthias.erb@ips.unibe.ch

Ian T. Baldwin (for transformed lines)
Tel: +49 3641 57 1100
Email: baldwin@ice.mpg.de

Summary

- Plants respond to herbivory by reconfiguring hormonal networks, increasing secondary metabolite production and decreasing growth. Furthermore, some plants display a decrease in leaf energy reserves in the form of soluble sugars and starch, leading to the hypothesis that herbivory-induced secondary metabolite production and growth reduction may be linked through a carbohydrate-based resource trade-off.

- In order to test the above hypothesis, we measured leaf carbohydrates and plant growth in seven genetically engineered *Nicotiana attenuata* genotypes that are deficient in one or several major herbivore-induced, jasmonate-dependent defensive secondary metabolites and proteins. Furthermore, we manipulated gibberellin and jasmonate signaling, and quantified the impact of these phytohormones on secondary metabolite production, sugar accumulation and growth.

- Simulated herbivore attack by *Manduca sexta* specifically reduced leaf sugar concentrations and growth in a jasmonate-dependent manner. These effects were similar or even stronger in defenseless genotypes with intact jasmonate signaling. Gibberellin complementation rescued carbohydrate accumulation and growth in induced plants without impairing the induction of defensive secondary metabolites.

- These results are consistent with a hormonal antagonism model rather than a resource–cost model to explain the negative relationship between herbivory-induced defenses, leaf energy reserves and growth.

Introduction

Trade-offs are fundamental to our understanding of evolution, as they define the trait space within which an organism can adapt to its environment. In simple terms, a trade-off refers to a situation in which one trait cannot increase without a decrease in another (Garland, 2014). Many trade-offs are due to limiting resources such as energy, space and time which cannot be used to increase the expression of more than one trait at once (de Jong & van Noordwijk, 1992; Garland, 2014).

Resource-based trade-offs are often employed as potential explanations for negative associations between different plant traits. When plants are attacked by herbivores, for instance, they start producing defensive metabolites and proteins and at the same time grow more slowly (Redman *et al.*, 2001; Ferrieri *et al.*, 2015). Under the assumption that the production of defenses and growth require the same limiting resources, the negative association between induced defenses and growth may be caused by a resource-based trade-off. However, as with many other negatively associated traits in nature (Bennett & Lenski, 2007), demonstrating that an

actual resource-based trade-off governs the negative relationship between induced defense and growth has remained challenging (Redman *et al.*, 2001; Huot *et al.*, 2014; Zhou *et al.*, 2015).

One approach to understand the connection between induced defense and suppressed growth is to identify the resource which may limit the simultaneous expression of both traits. Induced defenses and growth both require energy, for instance. Energy is captured through photosynthesis, transported to sink tissues in the form of sucrose, which is cleaved to glucose and fructose and used for glycolysis, and stored as starch (Braun *et al.*, 2014). Several studies show that upon herbivore attack, sugar and starch concentrations decrease in leaves (Babst *et al.*, 2005; van Dam & Oomen, 2008; Hanik *et al.*, 2010; Sampedro *et al.*, 2011; Machado *et al.*, 2013, 2015; Tytgat *et al.*, 2013; Ferrieri *et al.*, 2015). This decrease could either be the result of increased use of sugars for induced plant defenses (Arnold *et al.*, 2004; Heiling *et al.*, 2010; Ferrieri *et al.*, 2013) and/or a decrease in photosynthesis (Zangerl *et al.*, 1997; Tang *et al.*, 2006; Nabity *et al.*, 2012). Based on these findings, it is possible that energy in the form of soluble sugars and starch may be a limiting resource that

decreases growth in plants which are induced to produce higher amounts of defensive metabolites.

Recent studies show that phytohormonal cross-talk is important to understand how induced defenses and growth responses to herbivory are regulated (Yang *et al.*, 2012; Heinrich *et al.*, 2013). Jasmonates (JAs), for instance, which are important regulators of induced defenses, deplete growth-limiting resources such as starch and sugars (Babst *et al.*, 2005; Hanik *et al.*, 2010; Machado *et al.*, 2013, 2015, 2016a, 2017) and reduce plant growth (Bonaventure *et al.*, 2007; Zhang & Turner, 2008; Sampedro *et al.*, 2011) by delaying cell cycle progression and cell proliferation (Zhang & Turner, 2008; Noir *et al.*, 2013). JAs, together with herbivory-induced transcription factors also negatively regulate gibberellin (GA) signaling (Kim *et al.*, 2011; Yang *et al.*, 2012; Heinrich *et al.*, 2013; Li *et al.*, 2015). GAs are important regulators of photosynthesis, carbohydrate metabolism and plant growth (Richards *et al.*, 2001; Biemelt *et al.*, 2004; Ueguchi-Tanaka *et al.*, 2007; Huerta *et al.*, 2008). Altering GA signaling significantly affects plant photosynthetic capacity through changes in the activity and content of ribulose-1,5-bisphosphate carboxylase, chlorophyll content and chloroplast biogenesis rates (Yuan & Xu, 2001; Tuna *et al.*, 2008; Jiang *et al.*, 2012), which is frequently accompanied by increases of both sucrose synthesis and nonstructural carbohydrate pools (Miyamoto *et al.*, 1993; Chen *et al.*, 1994; Mehouachi *et al.*, 1996; Ranwala & Miller, 2008). Thus, the induction of JA and the simultaneous suppression of GA is likely to increase plant defenses and reduce plant growth in herbivore-attacked plants (Yang *et al.*, 2012; Heinrich *et al.*, 2013; Li *et al.*, 2015). Whether carbohydrate depletion in herbivory-attacked plants is the direct result of hormonal signaling or the indirect consequence of energy depletion by herbivore-induced defenses is unclear.

Phytohormonal cross-talk is not incompatible with the resource-based trade-off model. Plants may have evolved the capacity to actively regulate the allocation of resources through phytohormones to avoid overdepletion and misallocation while at the same time still being subject to a resource constraint (Huot *et al.*, 2014). Nevertheless, the insights into the regulatory pathways which govern induced defenses and growth suppression responses allow for the effective manipulation of the system (Heinrich *et al.*, 2013; Campos *et al.*, 2016) and thus to test whether plants are indeed constrained in their capacity to increase both traits simultaneously. A recent study in *Arabidopsis thaliana* shows that the parallel relief of transcriptional repression of defense- and growth-related signaling pathways in the mutant *jazQ phyB* results in herbivore-resistant plants with high secondary metabolite concentrations which grow big rosettes at the same time (Campos *et al.*, 2016). Moreover, by using JA-derivatives, growth and defense were uncoupled in wild tobacco (Jimenez-Aleman *et al.*, 2017).

In this study, we studied the association between herbivore-induced defensive metabolites and proteins, available energy in the form of sugars and starch as a potential limiting resource, and plant growth. As a null model, we assumed that herbivory induces defensive metabolites and proteins, and thereby reduces sugar and starch pools, and as a consequence plant growth. As

an alternative model, we postulated that sugars and starch pools and growth may be suppressed through induced hormonal signaling directly. To test these assumptions, we measured sugars and starch in seven genetically engineered *Nicotiana attenuata* plant genotypes that are impaired in the biosynthesis of one or several inducible defensive metabolites and proteins. To test for the effect of induced hormonal signaling on sugars, starch, growth and defensive metabolites, we manipulated JA and GA signaling using genetic and pharmacological approaches. Our results suggest that herbivore-induced hormonal cross-talk suppresses growth and carbohydrate accumulation independently of the production of defensive metabolites.

Materials and Methods

Plant cultivation

Seeds of different *Nicotiana attenuata* Torr. Ex. Watson plant genotypes were germinated on Gamborg's B5 medium as described previously (Krügel *et al.*, 2002). Nine to 10 d later, seedlings were transferred to Teku pots (Teku JP3050/104T, Pöppelmann GmbH & Co. KG, Lohne, Germany) for 10–12 d before transferring them into 1 l pots filled with soil. All plants were grown at 45–55% relative humidity and 23–25°C during days and 19–23°C during nights under 16 h of light (06:00–22:00 h). Plants were watered daily by a flood irrigation system. The characteristics of the transgenic plants used in this study are described in Tables 1 and 2.

Creation of defense-impaired genotypes

irPI/PMT*irGGPPS and irPI/PMT*irMYB8 lines were created by removing anthers from flowers of irPI/PMT plants (A-04-103-3-2) before pollen maturation and pollinating the stigmas with pollen from either irGGPPS (A-07-230-5-2) or irMYB8 (A-07-810) plants. The other plant genotypes were created in previous studies (Table 1).

Herbivory

Manduca sexta attack was simulated as described previously (Machado *et al.*, 2013). In brief, a pattern wheel was rolled six times on the leaf surface of three rosette leaves of 35-d-old plants and the wounds (W) were immediately treated with either water (W+W) or *M. sexta* oral secretions (W+OS) ($n = 5$). These treatments were carried out every other day for three times over a 6-d period. W+OS treatment results in plant responses that are very similar to real *M. sexta* attack (Schittko *et al.*, 2000; Qu *et al.*, 2004; Roda *et al.*, 2004; Giri *et al.*, 2006; Machado *et al.*, 2015, 2016b) and allowed us to standardize damage and induction across different genotypes.

Phytohormonal treatments

Either 150 µg methyl jasmonic acid (MeJA), 150 µg MeJA and 5 µg gibberellic acid (MeJA+GA₃), or 150 µg MeJA and 5 µg of

Table 1 Characteristics of the *Nicotiana attenuata* inverted repeat (ir) transgenic lines used in the present study

Genotype	Gene(s) silenced	Phenotype	Reference
irPI	Trypsin protease inhibitor (PI)	Reduced constitutive and herbivory-induced trypsin protease inhibitor activity	Zavala *et al.* (2004)
irPMT	Putrescine N-methyl transferase (PMT)	Reduced constitutive and herbivory-induced nicotine	Steppuhn *et al.* (2004)
irGGPPS	Geranylgeranyl pyrophosphate synthases (GGPPS)	Reduced constitutive and herbivory-induced 17-hydroxygeranyllinalool diterpene glycosides (DTGs)	Heiling *et al.* (2010)
irMYB8	R2R3-MYB8 transcription factor	Reduced constitutive and herbivory-induced phenolamides	Kaur *et al.* (2010)
irPI/PMT	Trypsin protease inhibitor (PI) and putrescine N-methyl transferase (PMT)	Reduced constitutive and herbivory-induced trypsin protease inhibitor activity and nicotine	Steppuhn & Baldwin (2007)
irPI/PMT*irGGPPS	PI, PMT and GGPPS	Reduced constitutive and herbivory-induced trypsin protease inhibitor activity, nicotine and DTGs	This study
irPI/PMT*irMYB8	PI, PMT and MYB8	Reduced constitutive and herbivory-induced trypsin protease inhibitor activity, nicotine and phenoalamides	This study

the gibberellin biosynthesis inhibitor uniconazole (MeJA+Uni) dissolved in lanolin paste were applied to the leaf base of three rosette leaves of 42-d-old plants. Control plants were treated with pure lanolin paste ($n = 5$). Applied doses were selected based on previous studies (Schomburg *et al.*, 2003; Heinrich *et al.*, 2013; Löfke *et al.*, 2013). No aberrant growth phenotypes were observed upon hormonal treatments.

Growth measurements

The stem length of all the plants was measured 5 and 6 d after induction, and plant growth rates were determined from the length increment. Stem length is a valid and robust estimate for plant dry mass in *N. attenuata* (Pandey *et al.*, 2008; Anssour *et al.*, 2009; Meldau *et al.*, 2012). We therefore used this parameter to nondestructively monitor the plant's investment into growth and to evaluate treatment effects during the most active growth phase (Mitra & Baldwin, 2008; Heinrich *et al.*, 2013).

Table 2 Metabolic profiles of the different *Nicotiana attenuata* genotypes used in this study

	TPIs	Nicotine	DTGs	PAs
EV	+	+	+	+
irPI		+	+	+
irPMT	+		+	+
irGGPPS	+	+		+
irMYB8	+	+	+	
irPI/PMT			+	+
irPI/PMT*irGGPPS				+
irPI/PMT*irMYB8			+	

+, capacity of the genotype to produce the metabolite; TPIs, trypsin protease inhibitor; DTGs, 17-hydroxygeranyllinalool diterpene glycosides; PAs, phenol amides; EV, empty vector. Characteristics of all genetically modified genotypes are shown in Table 1.

Primary and secondary metabolite quantifications

Treated leaves were harvested 6 d after induction and analyzed for glucose, fructose, sucrose and starch as described previously (Machado *et al.*, 2013, 2015). In short, plant tissues were harvested, flash frozen and ground to a fine powder in liquid nitrogen. One hundred milligrams of the resulting plant material were then extracted using 80% ethanol, followed by an incubation step (15 min at 80°C). The supernatant was removed and the remaining pellets were re-extracted twice with 50% ethanol (15 min at 80°C). All supernatants were pooled together, and glucose, fructose and sucrose were quantified enzymatically as described elsewhere (Velterop & Vos, 2001). The remaining pellets were used for an enzymatic determination of starch (Smith & Zeeman, 2006). Secondary metabolites were measured as described (Gaquerel *et al.*, 2010; Ferrieri *et al.*, 2015; Jimenez-Aleman *et al.*, 2015). For this, treated leaves were harvested 6 d after induction, flash frozen and ground to a fine powder in liquid nitrogen. Samples were extracted in 40% methanol and separated using a Rapid Separation LC (RSLC) system (Dionex Corp., Sunnyvale, CA, USA). A time-of-flight mass spectrometer equipped with an electrospray ionization source (Bruker Daltonic, Bremen, Germany) was used to determine the molecular mass of ionized molecular fragments and the amounts of the eluted analytes (Gaquerel *et al.*, 2010; Ferrieri *et al.*, 2015).

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using SIGMA PLOT 12.0 (Systat Software Inc., San Jose, CA, USA). Normality and equality of variance were verified using Shapiro–Wilk and Levene's tests, respectively. Holm–Sidak *post hoc* tests were used for multiple comparisons. Datasets from experiments that did not fulfill the assumptions for

ANOVA were natural log-, root square- or rank-transformed before analysis.

Results

Herbivory-induced jasmonates reduce leaf carbon and energy reserves

In order to determine whether herbivory-induced jasmonates (JAs) reduce leaf sugar and starch concentrations, we measured glucose, fructose and sucrose in wild-type (WT; EV, empty vector) and JA-deficient inverted repeat allene oxide cyclase (irAOC) plants upon simulated *M. sexta* attack. Similar to our previous study (Machado *et al.*, 2013), wounding (W+W) reduced glucose and fructose concentrations in WT plants, and these effects were further amplified by applying *M. sexta* oral secretions to the wounds (W+OS) (Fig. 1a,b). By contrast, W+W and W+OS treatments had no measurable impact on glucose and fructose concentrations in JA-deficient irAOC plants, whereas exogenous MeJA depleted glucose and fructose in both EV and irAOC plants. Sucrose pools were not significantly altered upon W+W and W+OS treatments in EV and irAOC plants (Fig. 1c). Sucrose concentrations were slightly reduced in MeJA-treated EV plants compared with controls and slightly higher in MeJA-treated irAOC plants compared with W+OS-treated irAOC plants.

The suppression of shoot growth and leaf carbon and energy reserves is independent of the induction of JA-dependent secondary metabolites

In order to test whether induced plant defenses impact leaf energy reserves and plant growth, we quantified leaf soluble sugars, starch and growth in seven different *N. attenuata* genotypes that are impaired in the production of one or several of the major JA-dependent secondary metabolites upon JA treatments (Table 2). In WT plants, MeJA treatment depleted glucose, fructose and starch pools and reduced plant growth (Fig. 2). The different defense-impaired genotypes also showed significantly reduced glucose, fructose and growth. In contrast to EV plants, sucrose pools were depleted in the different transgenic lines compared with WT plants (Fig. 2). In most cases, constitutive and MeJA-induced sugar and starch pools as well as growth did not differ between WT and transgenic genotypes (Supporting Information Table S1). IrPMT and irPI/PMT lines had higher leaf sucrose concentrations, and irGGPS plants showed a stronger suppression of sucrose upon MeJA treatment (Table S1).

Jasmonates constrain the accumulation of leaf carbon and energy and plant growth by antagonizing gibberellin signaling

Given that giberellins (GAs) are important regulators of photosynthesis, carbon metabolism and plant growth (Biemelt *et al.*, 2004; Davière & Achard, 2013) and that JA-signalling antagonizes this phytohormonal pathway (Heinrich *et al.*, 2013),
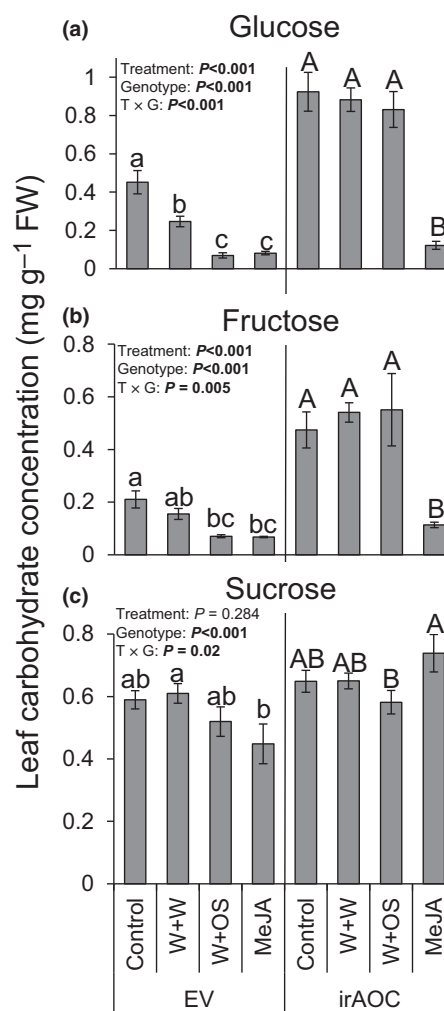

Fig. 1 Simulated herbivore attack depletes leaf soluble sugars in *Nicotiana attenuata* leaves in a jasmonate (JA)-dependent manner. Average (± SE) leaf (a) glucose, (b) fructose and (c) sucrose in response to simulated *Manduca sexta* attack in wild-type (EV, empty vector) and JA-biosynthesis deficient allene oxide cyclase (irAOC) plants ($n = 5$). Different letters indicate significant differences between treatments within genotypes ($P < 0.05$). Control, intact plants; W+W, wounding and water-treated plants; W+OS, wounded and *M. sexta* oral secretion-treated plants; MeJA, methyl jasmonate-treated plants.

we hypothesized that JAs may constrain plant carbon and energy accumulation and plant growth by interfering with GA biosynthesis. To test this hypothesis, we treated EV plants with methyl jasmonic acid (MeJA), gibberellic acid 3 (MeJA+GA$_3$) or uniconazole (MeJA+Uni) and measured leaf soluble sugars, starch and plant growth. GA$_3$ is an active gibberellin that restores WT phenotypes in gibberellin-biosynthesis-impaired plants (Yamaguchi, 2008). Uniconazole inhibits gibberellin biosynthesis by specifically blocking the cytochrome P450-mediated oxidation of *ent*-kaurene, a precursor of bioactive gibberellins (Izumi *et al.*, 1984, 1985; Katagi *et al.*, 1987; Lee *et al.*, 1998; Rademacher, 2000). On the one hand, GA$_3$ supplementation fully counteracted the JA-mediated depletion of plant carbohydrates and plant growth (Fig. 3). Uniconazol treatments, on the other, amplified the MeJA-induced reduction of glucose, sucrose and starch.
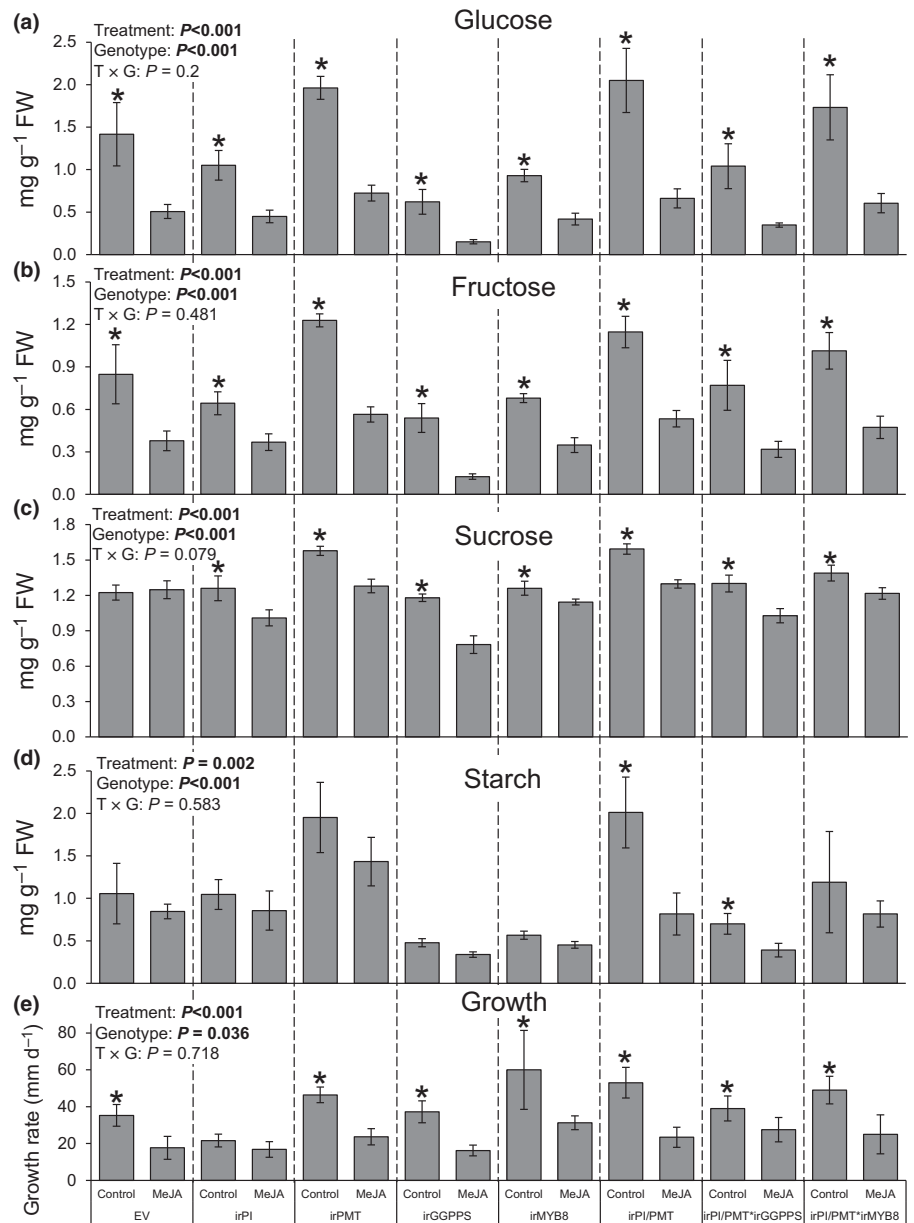
Fig. 2 Silencing the production of jasmonate (JA)-dependent secondary metabolites in *Nicotiana attenuata* does not alter the JA-induced carbohydrate depletion or growth suppression. Average ($\pm$ SE) leaf (a) glucose, (b) fructose, (c) sucrose, and (d) starch and (e) stem growth rates of plant genotypes with reduced biosynthetic capacity of JA-dependent secondary metabolites treated or not with methyl jasmonate (MeJA; $n = 5$). Asterisks indicate significant differences between MeJA-treated plants and controls within genotypes ($P < 0.05$). For additional statistical comparisons see Supporting Information Table S1. Control, lanolin-treated plants.

Fructose concentrations and growth were reduced to a similar extent than in MeJA-treated plants (Fig. 3). To test whether these effects are associated with changes in plant defenses, we measured plant secondary metabolites in MeJA-, GA$_3$- and uniconazole-treated plants. MeJA alone and in combination with either GA$_3$ or uniconazole induced defense metabolites to a similar extent (Fig. 4).

Discussion

Our experiments suggest that herbivory-induced jasmonates (JAs) impair the accumulation of leaf carbohydrates and plant growth through hormonal cross-talk rather than through the allocation of sugars and starch to the production of plant secondary metabolites.

Similar to our previous studies, we observed that simulated *Manduca sexta* attack strongly reduces leaf sugar accumulation (Machado *et al.*, 2013, 2015, 2016a). This effect was absent in JA-deficient genotypes and restored through JA complementation, indicating that JAs are herbivory-induced signals that negatively regulate sugar and starch accumulation in *Nicotiana attenuata*. JAs may negatively regulate sugar accumulation directly through hormonal cross-talk (see below), or indirectly by promoting the production of plant defenses such as secondary metabolites and defensive proteins. We found no evidence for a direct link between secondary metabolite and defensive protein induction, and sugar and starch pool depletion: JAs triggered the depletion of leaf carbohydrates and reduced plant growth rates in transgenic genotypes impaired in the synthesis of JA-dependent secondary metabolites to a similar or event greater extent than in wild-type plants. At
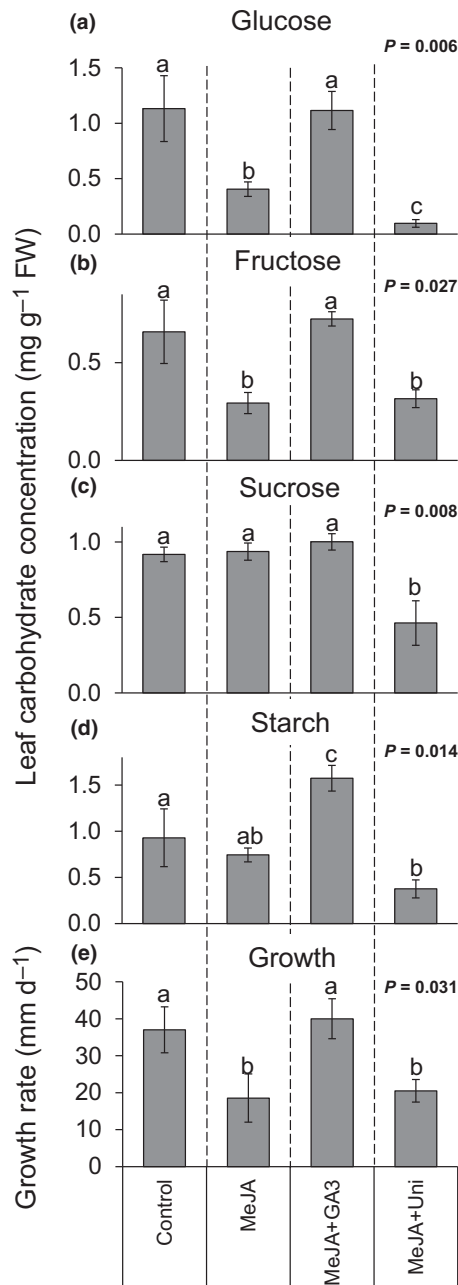
Fig. 3 Gibberellin complementation counteracts the jasmonate-induced sugar depletion and growth suppression in *Nicotiana attenuata*. Average (± SE) leaf (a) glucose, (b) fructose, (c) sucrose, and (d) starch and (e) stem growth rates following lanolin (control), methyl jasmonic acid (MeJA), MeJA + gibberellic acid 3 (GA₃) or MeJA + uniconazole (Uni) treatments (*n* = 5). Different letters indicate significant differences between treatments (*P* < 0.05). Control, lanolin-treated plants.

Fig. 4 Gibberellin complementation does not influence the jasmonate-mediated induction of secondary metabolites in *Nicotiana attenuata*. Average (± SE) leaf (a) caffeoylputrescine, (b) dicafeoylspermidine, (c) nicotine, and (d) 17-hydroxygeranyllinalool diterpene glycosides (DTGs) following lanolin (control), methyl jasmonic acid (MeJA), MeJA + gibberellic acid 3 (GA₃) or MeJA + uniconazole (Uni) treatments (*n* = 5). Different letters indicate significant differences between treatments (*P* < 0.05). Control, lanolin-treated plants.

present, we cannot exclude the possibility that other JA-inducible defensive phenotypes which we did not manipulate in our experiments act as energy sinks which reduce sugar and starch pools upon herbivore attack.

Earlier studies showed that *M. sexta* attack rapidly downregulates the expression of gibberellin biosynthetic genes and upregulates transcription factors that induce JA and decrease GA pools (Skibbe *et al.*, 2008; Kim *et al.*, 2011). Furthermore, JA-
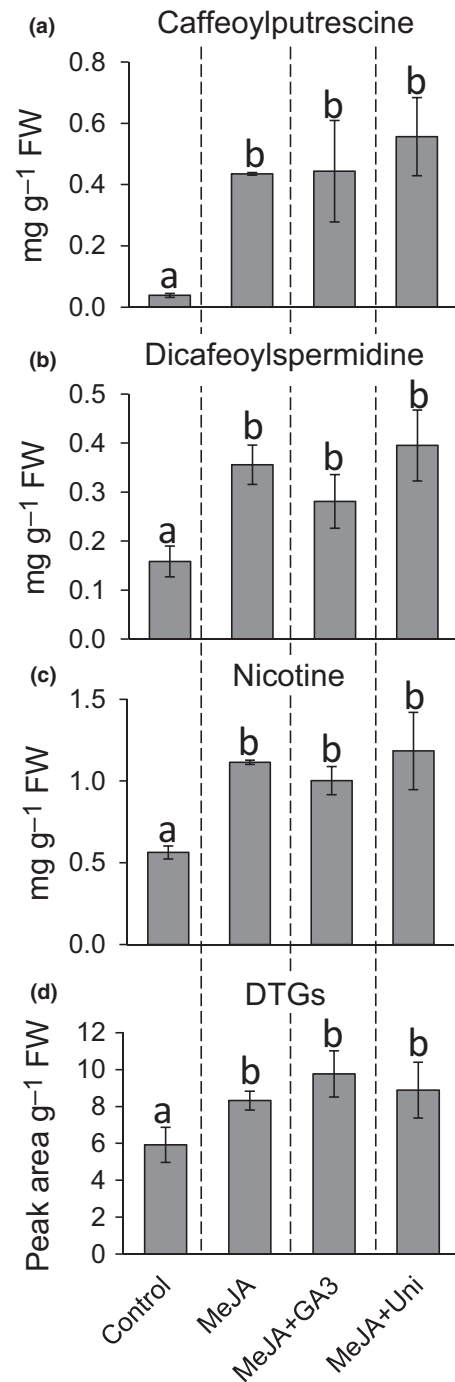
overproducing plants are impaired in GA biosynthesis and growth (Yang *et al.*, 2012; Heinrich *et al.*, 2013; Li *et al.*, 2015). Given that GAs are important regulators of photosynthesis, carbon metabolism and plant growth and that herbivory-induced JAs modulate this signaling pathway, we hypothesized that the

observed herbivory-induced suppression of sugars, starch and growth might be linked through these two hormones. Consistent with this hypothesis, we found that supplementing MeJA-induced plants with bioactive GAs fully counteracted both the JA-mediated carbohydrate depletion and the suppression of plant growth. Restoring leaf sugar and starch concentrations as well as growth through GA application was possible without impairing the production of induced defense metabolites. Because induced defense metabolite production can be uncoupled from the depletion of sugars and starch and the suppression of growth, we infer that sugars and starch do not act as a limiting resources which mediate a trade-off between induced secondary metabolites and growth.

From a mechanistic point of view, we propose that JA and GA signaling may reduce leaf carbohydrate accumulation by regulating photosynthesis. GAs potentiate photosynthetic capacity and carbon metabolism by promoting chlorophyll synthesis, increasing the activity and protein abundance of photosynthetic enzymes, and by increasing chloroplast biogenesis (Miyamoto *et al.*, 1993; Chen *et al.*, 1994; Mehouachi *et al.*, 1996; Fernandez *et al.*, 1997; Ghorbanli *et al.*, 2000; Yuan & Xu, 2001; Ashraf *et al.*, 2002; Afroz *et al.*, 2006; Shah, 2007; Ranwala & Miller, 2008; Sakamoto *et al.*, 2008; Tuna *et al.*, 2008; Jiang *et al.*, 2012). Herbivory-induced JAs induced opposite effects (Shan *et al.*, 2011; Nabity *et al.*, 2012; Machado *et al.*, 2013; Attaran *et al.*, 2014). Given that JAs reduce GA biosynthesis, it is likely that the negative effects of JAs on photosynthetic capacity occur indirectly through GA signaling. The reduced capacity of induced plants to produce photosynthates may then lead to a reduction in growth. Further experiments involving well-resolved measurements of photosynthetic rates, carbohydrate concentrations together with the genetic manipulation of JA and/or GA signaling pathways will help to better understand the exact mechanisms through which these two plant signals regulate energy accumulation and plant growth under herbivore pressure.

In our experiments, the induction of plant secondary metabolites and proteins *per se* did not significantly influence soluble sugar pools. Given that the production of plant secondary metabolites demands energy and carbon skeletons, it is frequently suggested that their induction should impact soluble sugar and starch accumulation (Machado *et al.*, 2013; Schultz *et al.*, 2013; Zhou *et al.*, 2015). The use of soluble proteins as a source of amino acids to fuel secondary metabolite production might be a plant strategy to minimize the sugar depletion to a point where other vital processes cannot be maintained (Baldwin, 1999; Ishihara *et al.*, 1999; Steppuhn *et al.*, 2004; Chen *et al.*, 2006; Kaur *et al.*, 2010; Tzin & Galili, 2010; Takano *et al.*, 2012; Kim *et al.*, 2013; Noge & Tamogami, 2013). Consistent with this hypothesis, recent studies demonstrate that herbivory-induced JAs reduce soluble protein content, which might reflect an increase demand for free amino acids (Ullmann-Zeunert *et al.*, 2013). Alternatively, lipids could also provide energy and carbon for secondary metabolite synthesis (Baena-González *et al.*, 2007; Chapman *et al.*, 2012). Although the plant lipidome responds to biotic and abiotic stress (Kallenbach *et al.*, 2010; Degenkolbe *et al.*, 2012; Marti *et al.*, 2013), it remains to be determined whether lipids can provide carbon for the synthesis of plant defenses (Welti &

Wang, 2004; Andreou *et al.*, 2009; Allmann *et al.*, 2010; Zhou *et al.*, 2015).

Nicotiana attenuata is not the only plant species in which growth and resistance traits can be uncoupled by reprogramming the plant's signaling network (Campos *et al.*, 2016; Jimenez-Aleman *et al.*, 2017). Using a genetic approach, it was recently demonstrated that rosette dry masses and herbivore resistance (measured as caterpillar growth suppression) can be boosted simultaneously in an Arabidopsis mutant which lacks five ZIM-domain transcriptional repressors as well as the photoreceptor phyB (Campos *et al.*, 2016). Our results furthermore suggest that uncoupling growth- and defense-related phenotypes is indeed possible at the metabolite level. Whether the reconstituted plants are able to resist herbivory, grow and finally reproduce normally under ecologically relevant conditions, however, remains to be determined.

Resource based trade-offs between growth and defense are often discussed as important factors which determine adaptive evolution in plants. Understanding and manipulating the links between defense and growth traits on a mechanistic level can help to test whether they are connected through the same limiting resources or whether their negative association is based on another mechanism. Using such an approach, we show that sugars and starch do not mediate the connection between the induction of defensive metabolites and the suppression of plant growth. Further research will be required to investigate the prevalence of resource-based trade-offs in the context of different environmental conditions, plant resources, defensive syndromes and fitness-relevant growth traits.

Author contributions

R.A.R.M. conceived the study, designed and carried out all the experiments, analyzed data and wrote the manuscript; M.E. analyzed data and wrote the manuscript; and I.T.B. contributed to writing the manuscript. All co-authors read and approved the final version of the manuscript.

References

Afroz S, Mohammad F, Hayat S, Siddiqui MH. 2006. Exogenous application of gibberellic acid counteracts the ill effect of sodium chloride in mustard. *Turkish Journal of Biology* 29: 233–236.

Allmann S, Halitschke R, Schuurink RC, Baldwin IT. 2010. Oxylipin channelling in *Nicotiana attenuata*: lipoxygenase 2 supplies substrates for green leaf volatile production. *Plant, Cell & Environment* 33: 2028–2040.

Andreou A, Brodhun F, Feussner I. 2009. Biosynthesis of oxylipins in non-mammals. *Progress in Lipid Research* 48: 148–170.

Anssour S, Krügel T, Sharbel TF, Saluz HP, Bonaventure G, Baldwin IT. 2009. Phenotypic, genetic and genomic consequences of natural and synthetic polyploidization of *Nicotiana attenuata* and *Nicotiana obtusifolia*. *Annals of Botany* 103: 1207–1217.

Arnold T, Appel H, Patel V, Stocum E, Kavalier A, Schultz J. 2004. Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink–source model of plant defense. *New Phytologist* 164: 157–164.

Ashraf M, Karim F, Rasul E. 2002. Interactive effects of gibberellic acid (GA3) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. *Plant Growth Regulation* 36: 49–59.

Attaran E, Major IT, Cruz JA, Rosa BA, Koo AJK, Chen J, Kramer DM, He SY, Howe GA. 2014. Temporal dynamics of growth and photosynthesis suppression in response to jasmonate signaling. *Plant Physiology* 165: 1302–1314.

Babst BA, Ferrieri RA, Gray DW, Lerdau M, Schlyer DJ, Schueller M, Thorpe MR, Orians CM. 2005. Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. *New Phytologist* 167: 63–72.

Baena-González E, Rolland F, Thevelein JM, Sheen J. 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature* 448: 938–942.

Baldwin IT. 1999. Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. *Journal of Chemical Ecology* 25: 3–30.

Bennett AF, Lenski RE. 2007. An experimental test of evolutionary trade-offs during temperature adaptation. *Proceedings of the National Academy of Sciences, USA* 104: 8649–8654.

Biemelt S, Tschiersch H, Sonnewald U. 2004. Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. *Plant Physiology* 135: 254–265.

Bonaventure G, Gfeller A, Proebsting WM, Hörtensteiner S, Chételat A, Martinoia E, Farmer EE. 2007. A gain of function allele of TPC1 activates oxylipin biogenesis after leaf wounding in Arabidopsis. *Plant Journal* 49: 889–898.

Braun DM, Wang L, Ruan Y-L. 2014. Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. *Journal of Experimental Botany* 65: 1713–1735.

Campos ML, Yoshida Y, Major IT, Oliveira Ferreira D, Weraduwage SM, Froehlich JE, Johnson BF, Kramer DM, Jander G, Sharkey TD *et al.* 2016. Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nature Communications* 7: 12570.

Chapman KD, Dyer JM, Mullen RT. 2012. Biogenesis and functions of lipid droplets in plants. Thematic review series: lipid droplet synthesis and metabolism: from yeast to man. *Journal of Lipid Research* 53: 215–226.

Chen H, Jones AD, Howe GA. 2006. Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. *FEBS Letters* 580: 2540–2546.

Chen W-S, Liu H-Y, Liu Z-H, Yang L, Chen W-H. 1994. Gibberellin and temperature influence carbohydrate content and flowering in *Phalaenopsis*. *Physiologia Plantarum* 90: 391–395.

van Dam NM, Oomen MW. 2008. Root and shoot jasmonic acid applications differentially affect leaf chemistry and herbivore growth. *Plant Signaling & Behavior* 3: 91–98.

Davière J-M, Achard P. 2013. Gibberellin signaling in plants. *Development* 140: 1147–1151.

Degenkolbe T, Giavalisco P, Zuther E, Seiwert B, Hincha DK, Willmitzer L. 2012. Differential remodeling of the lipidome during cold acclimation in natural accessions of *Arabidopsis thaliana*. *Plant Journal* 72: 972–982.

Fernandez JA, Banon S, Franco JA, González A, Martinez PF. 1997. Effects of vernalization and exogenous gibberellins on curd induction and carbohydrate levels in the apex of cauliflower (*Brassica oleracea* var. botrytis). *Scientia Horticulturae* 70: 223–230.

Ferrieri AP, Agtuca B, Appel HM, Ferrieri RA, Schultz JC. 2013. Temporal changes in allocation and partitioning of new carbon as ^{11}C elicited by simulated herbivory suggest that roots shape aboveground responses in Arabidopsis. *Plant Physiology* 161: 692–704.

Ferrieri AP, Arce CCM, Machado RAR, Meza-Canales ID, Lima E, Baldwin IT, Erb M. 2015. A *Nicotiana attenuata* cell wall invertase inhibitor (NaCWII) reduces growth and increases secondary metabolite biosynthesis in herbivore-attacked plants. *New Phytologist* 208: 519–530.

Gaquerel E, Heiling S, Schöttner M, Zurek G, Baldwin IT. 2010. Development and validation of a liquid chromatography− electrospray ionization− time-of-flight mass spectrometry method for induced changes in *Nicotiana attenuata* leaves during simulated herbivory. *Journal of Agricultural and Food Chemistry* 58: 9418–9427.

Garland T. 2014. Trade-offs. *Current Biology* 24: R60–R61.

Ghorbanli M, Kaveh SH, Sepehr MF. 2000. Effects of cadmium and gibberellin on growth and photosynthesis of *Glycine max*. *Photosynthetica* 37: 627–631.

Giri AP, Wünsche H, Mitra S, Zavala JA, Muck A, Svatoš Baldwin IT. 2006. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VII. Changes in the plant's proteome. *Plant Physiology* 142: 1621–1641.

Hanik N, Gómez S, Best M, Schueller M, Orians CM, Ferrieri RA. 2010. Partitioning of new carbon as ^{11}C in *Nicotiana tabacum* reveals insight into methyl jasmonate induced changes in metabolism. *Journal of Chemical Ecology* 36: 1058–1067.

Heiling S, Schuman MC, Schoettner M, Mukerjee P, Berger B, Schneider B, Jassbi AR, Baldwin IT. 2010. Jasmonate and ppHsystemin regulate key malonylation steps in the biosynthesis of 17-hydroxygeranyllinalool diterpene glycosides, an abundant and effective direct defense against herbivores in *Nicotiana attenuata*. *Plant Cell* 22: 273–292.

Heinrich M, Hettenhausen C, Lange T, Wünsche H, Fang J, Baldwin IT, Wu J. 2013. High levels of jasmonic acid antagonize the biosynthesis of gibberellins and inhibit the growth of *Nicotiana attenuata* stems. *Plant Journal* 73: 591–606.

Huerta L, Forment J, Gadea J, Fagoaga C, Peňa L, Pérez-Amador MA, García-Martínez JL. 2008. Gene expression analysis in citrus reveals the role of gibberellins on photosynthesis and stress. *Plant, Cell & Environment* 31: 1620–1633.

Huot B, Yao J, Montgomery BL, He SY. 2014. Growth–defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant* 7: 1267–1287.

Ishihara A, Ohtsu Y, Iwamura H. 1999. Biosynthesis of oat avenanthramide phytoalexins. *Phytochemistry* 50: 237–242.

Izumi K, Kamiya Y, Sakurai A, Oshio H, Takahashi N. 1985. Studies of sites of action of a new plant growth retardant (E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol (S-3307) and comparative effects of its stereoisomers in a cell-free system from *Cucurbita maxima*. *Plant and Cell Physiology* 26: 821–827.

Izumi K, Yamaguchi I, Wada A, Oshio H, Takahashi N. 1984. Effects of a new plant growth retardant (E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol (S-3307) on the growth and gibberellin content of rice plants. *Plant and Cell Physiology* 25: 611–617.

Jiang X, Li H, Wang T, Peng C, Wang H, Wu H, Wang X. 2012. Gibberellin indirectly promotes chloroplast biogenesis as a means to maintain the chloroplast population of expanded cells. *Plant Journal* 72: 768–780.

Jimenez-Aleman GH, Machado RAR, Baldwin IT, Boland W. 2017. JA-Ile-macrolactones uncouple growth and defense in wild tobacco. *Organic & Biomolecular Chemistry* 15: 3391–3395.

Jimenez-Aleman GH, Machado RAR, Görls H, Baldwin IT, Boland W. 2015. Synthesis, structural characterization and biological activity of two diastereomeric JA-Ile macrolactones. *Organic & Biomolecular Chemistry* 13: 5885–5893.

de Jong G, van Noordwijk AJ. 1992. Acquisition and allocation of resources: genetic (co) variances, selection, and life histories. *American Naturalist* 139: 749–770.

Kallenbach M, Alagna F, Baldwin IT, Bonaventure G. 2010. *Nicotiana attenuata* SIPK, WIPK, NPR1, and fatty acid-amino acid conjugates participate in the induction of jasmonic acid biosynthesis by affecting early enzymatic steps in the pathway. *Plant Physiology* 152: 96–106.

Katagi T, Mikami N, Matsuda T, Miyamoto J. 1987. Structural studies of the plant growth regulator uniconazole (ES pure) and computer-aided analysis of its interaction with cytochrome P-450. *Journal of Pesticide Science* **12**: 627–633.

Kaur H, Heinzel N, Schöttner M, Baldwin IT, Gális I. 2010. R2R3-NaMYB8 regulates the accumulation of phenylpropanoid-polyamine conjugates, which are essential for local and systemic defense against insect herbivores in *Nicotiana attenuata*. *Plant Physiology* **152**: 1731–1747.

Kim S-G, Yon F, Gaquerel E, Gulati J, Baldwin IT. 2011. Tissue specific diurnal rhythms of metabolites and their regulation during herbivore attack in a native tobacco, *Nicotiana attenuata*. *PLoS ONE* **6**: e26214.

Kim YB, Kim JK, Uddin MR, Xu H, Park WT, Tuan PA, Li X, Chung E, Lee JH, Park SU. 2013. Metabolomics analysis and biosynthesis of rosmarinic acid in *Agastache rugosa* Kuntze treated with methyl jasmonate. *PLoS ONE* **8**: e64199.

Krügel T, Lim M, Gase K, Halitschke R, Baldwin IT. 2002. *Agrobacterium*-mediated transformation of *Nicotiana attenuata*, a model ecological expression system. *Chemoecology* **12**: 177–183.

Lee I-J, Foster KR, Morgan PW. 1998. Effect of gibberellin biosynthesis inhibitors on native gibberellin content, growth and floral initiation in *Sorghum bicolor*. *Journal of Plant Growth Regulation* **17**: 185–195.

Li R, Zhang J, Li J, Zhou G, Wang Q, Bian W, Erb M, Lou Y. 2015. Prioritizing plant defence over growth through WRKY regulation facilitates infestation by non-target herbivores. *eLife* **4**: e04805.

Löfke C, Zwiewka M, Heilmann I, van Montagu MCE, Teichmann T, Friml J. 2013. Asymmetric gibberellin signaling regulates vacuolar trafficking of PIN auxin transporters during root gravitropism. *Proceedings of the National Academy of Sciences, USA* **110**: 3627–3632.

Machado RAR, Arce C, Ferrieri AP, Baldwin IT, Erb M. 2015. Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*. *New Phytologist* **207**: 91–105.

Machado RAR, Ferrieri AP, Robert CAM, Glauser G, Kallenbach M, Baldwin IT, Erb M. 2013. Leaf–herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytologist* **200**: 1234–1246.

Machado RAR, McClure M, Baldwin IT, Erb M. 2016a. Benefits of jasmonate-dependent defenses against vertebrate herbivores in nature. *eLife* **5**: e13720.

Machado RAR, Robert CAM, Arce CCM, Ferrieri AP, Xu S, Jimenez-Aleman GH, Baldwin IT, Erb M. 2016b. Auxin is rapidly induced by herbivory attack and regulates systemic, jasmonate-dependent defenses. *Plant Physiology* **172**: 521–532.

Machado RAR, Zhou W, Ferrieri AP, Arce CCM, Baldwin IT, Xu S, Erb M. 2017. Species specific regulation of herbivory induced defoliation tolerance is associated with jasmonate inducibility. *Ecology and Evolution*. doi: 10.1002/ece3.2953.

Marti G, Erb M, Boccard J, Glauser G, Doyen GR, Villard N, Robert CAM, Turlings TCJ, Rudaz S, Wolfender J-L. 2013. Metabolomics reveals herbivore–induced metabolites of resistance and susceptibility in maize leaves and roots. *Plant, Cell & Environment* **36**: 621–639.

Mehouachi J, Tadeo FR, Zaragoza S, Primo-Millo E, Talon M. 1996. Effects of gibberellic acid and paclobutrazol on growth and carbohydrate accumulation in shoots and roots of citrus rootstock seedlings. *Journal of Horticultural Science* **71**: 747–754.

Meldau S, Ullman-Zeunert L, Govind G, Bartram S, Baldwin IT. 2012. MAPK-dependent JA and SA signalling in *Nicotiana attenuata* affects plant growth and fitness during competition with conspecifics. *BMC Plant Biology* **12**: 213.

Mitra S, Baldwin IT. 2008. Independently silencing two photosynthetic proteins in *Nicotiana attenuata* has different effects on herbivore resistance. *Plant Physiology* **148**: 1128–1138.

Miyamoto K, Ueda J, Kamisaka S. 1993. Gibberellin–enhanced sugar accumulation in growing subhooks of etiolated *Pisum sativum* seedlings. Effects of gibberellic acid, indoleacetic acid and cycloheximide on invertase activity, sugar accumulation and growth. *Physiologia Plantarum* **88**: 301–306.

Nabity PD, Zavala JA, DeLucia EH. 2012. Herbivore induction of jasmonic acid and chemical defences reduce photosynthesis in *Nicotiana attenuata*. *Journal of Experimental Botany* **64**: 685–694.

Noge K, Tamogami S. 2013. Herbivore-induced phenylacetonitrile is biosynthesized from *de novo*-synthesized l-phenylalanine in the giant knotweed, *Fallopia sachalinensis*. *FEBS Letters* **587**: 1811–1817.

Noir S, Bömer M, Takahashi N, Ishida T, Tsui T-J, Balbi V, Shanahan H, Sugimoto K, Devoto A. 2013. Jasmonate controls leaf growth by repressing cell proliferation and the onset of endoreduplication while maintaining a potential stand-by mode. *Plant Physiology* **161**: 1930–1951.

Pandey SP, Gaquerel E, Gase K, Baldwin IT. 2008. *RNA-directed RNA polymerase3* from *Nicotiana attenuata* is required for competitive growth in natural environments. *Plant Physiology* **147**: 1212–1224.

Qu N, Schittko U, Baldwin IT. 2004. Consistency of *Nicotiana attenuata*'s herbivore-and jasmonate-induced transcriptional responses in the allotetraploid species *Nicotiana quadrivalvis* and *Nicotiana clevelandii*. *Plant Physiology* **135**: 539–548.

Rademacher W. 2000. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Biology* **51**: 501–531.

Ranwala AP, Miller WB. 2008. Gibberellin-mediated changes in carbohydrate metabolism during flower stalk elongation in tulips. *Plant Growth Regulation* **55**: 241–248.

Redman AM, Cipollini DF Jr, Schultz JC. 2001. Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. *Oecologia* **126**: 380–385.

Richards DE, King KE, Ait-ali T, Harberd NP. 2001. How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. *Annual Review of Plant Biology* **52**: 67–88.

Roda AM, Halitschke R, Steppuhn A, Baldwin IT. 2004. Individual variability in herbivore–specific elicitors from the plant's perspective. *Molecular Ecology* **13**: 2421–2433.

Sakamoto W, Miyagishima S-y, Jarvis P. 2008. Chloroplast biogenesis: control of plastid development, protein import, division and inheritance. *Arabidopsis Book* **6**: e0110.

Sampedro L, Moreira X, Zas R. 2011. Costs of constitutive and herbivore–induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology* **99**: 818–827.

Schittko U, Hermsmeier D, Baldwin IT. 2000. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. II. Accumulation of plant mRNAs in response to insect-derived cues. *Plant Physiology* **125**: 701–710.

Schomburg FM, Bizzell CM, Lee DJ, Zeevaart JAD, Amasino RM. 2003. Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. *Plant Cell* **15**: 151–163.

Schultz JC, Appel HM, Ferrieri AP, Arnold TM. 2013. Flexible resource allocation during plant defense responses. *Frontiers in Plant Science* **4**: 324.

Shah SH. 2007. Effects of salt stress on mustard as affected by gibberellic acid application. *General and Applied Plant Physiology* **33**: 97–106.

Shan X, Wang J, Chua L, Jiang D, Peng W, Xie D. 2011. The role of Arabidopsis Rubisco activase in jasmonate-induced leaf senescence. *Plant Physiology* **155**: 751–764.

Skibbe M, Qu N, Galis I, Baldwin IT. 2008. Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. *Plant Cell* **20**: 1984–2000.

Smith AM, Zeeman SC. 2006. Quantification of starch in plant tissues. *Nature Protocols* **1**: 1342–1345.

Steppuhn A, Baldwin IT. 2007. Resistance management in a native plant: nicotine prevents herbivores from compensating for plant protease inhibitors. *Ecology Letters* **10**: 499–511.

Steppuhn A, Gase K, Krock B, Halitschke R, Baldwin IT. 2004. Nicotine's defensive function in nature. *PLoS Biology* **2**: 1074–1080.

Takano A, Kakehi J-I, Takahashi T. 2012. Thermospermine is not a minor polyamine in the plant kingdom. *Plant and Cell Physiology* **53**: 606–616.

Tang JY, Zielinski RE, Zangerl AR, Crofts AR, Berenbaum MR, DeLucia EH. 2006. The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. *Journal of Experimental Botany* **57**: 527–536.

Tuna AL, Kaya C, Dikilitas M, Higgs D. 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany* **62**: 1–9.

New
Phytologist

Tytgat TOG, Verhoeven KJF, Jansen JJ, Raaijmakers CE, Bakx-Schotman T, McIntyre LM, van der Putten WH, Biere A, van Dam NM. 2013. Plants know where it hurts: root and shoot jasmonic acid induction elicit differential responses in *Brassica oleracea*. *PLoS ONE* 8: e65502.

Tzin V, Galili G. 2010. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Molecular Plant* 3: 956–972.

Ueguchi-Tanaka M, Nakajima M, Motoyuki A, Matsuoka M. 2007. Gibberellin receptor and its role in gibberellin signaling in plants. *Annual Review of Plant Biology* 58: 183–198.

Ullmann-Zeunert L, Stanton MA, Wielsch N, Bartram S, Hummert C, Svatoš A, Baldwin IT, Groten K. 2013. Quantification of growth–defense trade–offs in a common currency: nitrogen required for phenolamide biosynthesis is not derived from ribulose–1, 5–bisphosphate carboxylase/oxygenase turnover. *Plant Journal* 75: 417–429.

Velterop JS, Vos F. 2001. A rapid and inexpensive microplate assay for the enzymatic determination of glucose, fructose, sucrose, L-malate and citrate in tomato (*Lycopersicon esculentum*) extracts and in orange juice. *Phytochemical Analysis* 12: 299–304.

Welti R, Wang X. 2004. Lipid species profiling: a high-throughput approach to identify lipid compositional changes and determine the function of genes involved in lipid metabolism and signaling. *Current Opinion in Plant Biology* 7: 337–344.

Yamaguchi S. 2008. Gibberellin metabolism and its regulation. *Annual Review of Plant Biology* 59: 225–251.

Yang D-L, Yao J, Mei C-S, Tong X-H, Zeng L-J, Li Q, Xiao L-T, T-P Sun, Li J, Deng X-W et al. 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences, USA* 109: E1192–E1200.

Yuan L, Xu D-Q. 2001. Stimulation effect of gibberellic acid short-term treatment on leaf photosynthesis related to the increase in Rubisco content in broad bean and soybean. *Photosynthesis Research* 68: 39–47.

Zangerl AR, Arntz AM, Berenbaum MR. 1997. Physiological price of an induced chemical defense: photosynthesis, respiration, biosynthesis, and growth. *Oecologia* 109: 433–441.

Zavala JA, Patankar AG, Gase K, Hui D, Baldwin IT. 2004. Manipulation of endogenous trypsin proteinase inhibitor production in *Nicotiana attenuata* demonstrates their function as antiherbivore defenses. *Plant Physiology* 134: 1181–1190.

Zhang YI, Turner JG. 2008. Wound-induced endogenous jasmonates stunt plant growth by inhibiting mitosis. *PLoS ONE* 3: e3699.

Zhou S, Lou Y-R, Tzin V, Jander G. 2015. Alteration of plant primary metabolism in response to insect herbivory. *Plant Physiology* 169: 1488–1498.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 *P*-values of Holm–Sidak multiple comparisons of carbohydrates and growth between different *Nicotiana attenuata* genotypes within treatments (data shown in Fig. 2)

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.

- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.

- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)

- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**