New Phytologist

Research

# Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*

**Ricardo A. R. Machado[1,2], Carla C. M. Arce[1,2,3], Abigail P. Ferrieri[1,2], Ian T. Baldwin[2] and Matthias Erb[1,4]**

[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]Department of Entomology, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, 36570-000 Viçosa, Brazil; [4]Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

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

Received: *4 December 2014*
Accepted: *18 January 2015*

*New Phytologist* (2015) **207:** 91–105
**doi**: 10.1111/nph.13337

**Key words:** carbohydrates, insect nutrition, jasmonates, *Manduca sexta*, *Nicotiana attenuata*, primary metabolism.

## Summary

- Jasmonates regulate plant secondary metabolism and herbivore resistance. How they influence primary metabolites and how this may affect herbivore growth and performance are not well understood.
- We profiled sugars and starch of jasmonate biosynthesis-deficient and jasmonate-insensitive *Nicotiana attenuata* plants and manipulated leaf carbohydrates through genetic engineering and *in vitro* complementation to assess how jasmonate-dependent sugar accumulation affects the growth of *Manduca sexta* caterpillars.
- We found that jasmonates reduce the constitutive and herbivore-induced concentration of glucose and fructose in the leaves across different developmental stages. Diurnal, jasmonate-dependent inhibition of invertase activity was identified as a likely mechanism for this phenomenon. Contrary to our expectation, both *in planta* and *in vitro* approaches showed that the lower sugar concentrations led to increased *M. sexta* growth. As a consequence, jasmonate-dependent depletion of sugars rendered *N. attenuata* plants more susceptible to *M. sexta* attack.
- In conclusion, jasmonates are important regulators of leaf carbohydrate accumulation and this determines herbivore growth. Jasmonate-dependent resistance is reduced rather than enhanced through the suppression of glucose and fructose concentrations, which may contribute to the evolution of divergent resistance strategies of plants in nature.

## Introduction

Jasmonates regulate plant responses to biotic and abiotic stress and influence plant growth and development. They are part of the regulatory networks of plant–symbiont (Pozo & Azcón-Aguilar, 2007; Stein *et al.*, 2008; Jacobs *et al.*, 2011), plant–pathogen (Landgraf *et al.*, 2012) and plant–herbivore interactions (reviewed by Wu & Baldwin, 2010), and are involved in the regulation of seed germination (Corbineau *et al.*, 1988), root growth and development (Staswick *et al.*, 1992), leaf movement (Nakamura *et al.*, 2006) and flower development (Li *et al.*, 2004). Perhaps the best known function of jasmonates is their stimulatory effect on plant secondary chemistry. Plants impaired in jasmonate production or perception generally display reduced levels of constitutive and induced secondary metabolites (Chen *et al.*, 2006; Paschold *et al.*, 2007; Shoji *et al.*, 2008; Zhang *et al.*, 2011).

Although our understanding of several aspects of jasmonate signaling is increasing, knowledge about its possible role as a regulator of primary metabolism in plants is unclear. Recently, leaf glucose and fructose concentrations were found to be constitutively higher and less depleted in response to simulated *Manduca sexta* herbivory in jasmonate biosynthesis-deficient *Nicotiana attenuata* plants, an effect that can be mimicked by the exogenous application of jasmonic acid (JA; Machado *et al.*, 2013). Moreover, exogenous jasmonate application to the leaves reduced leaf starch concentration in poplar trees, stem sugars in tulip, leaf sugars in tobacco, and leaf sugars and amino acids in cabbage (Babst *et al.*, 2005; Skrzypek *et al.*, 2005; van Dam & Oomen, 2008; Hanik *et al.*, 2010; Tytgat *et al.*, 2013), suggesting that jasmonates might act as negative regulators of plant primary metabolism. By contrast, starch concentrations in jasmonate signaling-impaired tobacco plants were significantly lower (Wang *et al.*, 2014) and jasmonate application to the leaves induced amino acids in tobacco leaves (Hanik *et al.*, 2010), suggesting that jasmonates can also promote starch and amino acid accumulation. A detailed analysis of primary metabolites in jasmonate signaling-impaired plants is therefore required to clarify the potential role of endogenous jasmonates in the regulation of plant primary metabolism. Constitutive and induced jasmonate levels change over plant development (Abdala *et al.*, 2002; Diezel *et al.*, 2011), a phenomenon that correlates with a reduction in the magnitude of induction of jasmonate-dependent secondary metabolites and defensive proteins (van Dam *et al.*, 2001; Kaur *et al.*, 2010;

Onkokesung *et al.*, 2012). It is therefore possible that the impact of jasmonates on leaf carbohydrates is dependent on a plant's developmental stage.

Sugars are the dominant soluble leaf carbohydrates of plants. They are produced through the incorporation of carbon dioxide ($CO_2$) into ribulose-1,5-bisphosphate (RuBP) by the action of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), followed by the spontaneous formation of two molecules of 3-phosphoglyceric acid (3PGA). RuBisCO activase (RCA) activates RuBisCO by removing inhibitory sugar phosphates from the active site. 3PGA is subsequently converted to glucose, fructose, sucrose and starch via several enzymatic steps. Sucrose and starch can be stored, transported and/or metabolized further (Braun *et al.*, 2014). Soluble sugars and starch accumulate during the day and are catabolized during the night to meet the energy demand of the plant. Therefore, their concentrations rise and fall in a diurnal manner. Diurnal patterns therefore need to be taken into account when studying the impact of jasmonates on leaf carbohydrates.

Phytophagous insects feed on plants to acquire nutrients to fuel growth, development and reproduction, and are therefore affected directly by the metabolic make up of their food source. Both primary and secondary metabolites influence insect performance (Roeder & Behmer, 2014). Secondary metabolites are directly toxic or reduce the digestibility of the plant material in a quantitative manner (Bennett & Wallsgrove, 1994). The influence of primary metabolites on herbivores is more context dependent (Behmer, 2008). The ratio between carbohydrates and protein, for instance, determines insect growth in a nonlinear fashion, with suboptimal ratios leading to a rapid reduction in growth rates (Thompson & Redak, 2000; Simpson & Raubenheimer, 2009; Roeder & Behmer, 2014). Furthermore, protein and carbohydrate ratios influence the toxicity of plant secondary metabolites (Raubenheimer & Simpson, 1990; Raubenheimer, 1992; Simpson & Raubenheimer, 2001). Most studies on insect nutrition have been carried out in chemically defined artificial environments. However, plants as food sources in nature are inherently variable. Herbivore attack, for instance, alters nitrogen and carbon dynamics (Arnold & Schultz, 2002; Babst *et al.*, 2005; Gómez *et al.*, 2010; Appel *et al.*, 2012), which often results in dramatic changes in primary and secondary metabolite pools (Babst *et al.*, 2005; Skrzypek *et al.*, 2005; Schwachtje *et al.*, 2006; Steinbrenner *et al.*, 2011; Gómez *et al.*, 2012; Machado *et al.*, 2013) that might affect the nutritional quality of foliar tissue and could potentially affect herbivore nutrition. If we are to understand the importance of carbohydrates for insect nutrition, combining *in vitro* assays with experiments *in planta* would therefore be a promising approach.

One approach to manipulate plant chemistry is to target defensive signals. Jasmonates, for instance, have been silenced in a number of plant species, and the susceptibility of the jasmonate signaling-impaired plants to herbivores has subsequently been attributed to deficiencies in secondary metabolite production and accumulation (Steppuhn *et al.*, 2004; Paschold *et al.*, 2007; Steppuhn & Baldwin, 2007; Heiling *et al.*, 2010). Given that jasmonates also regulate primary metabolites in plants (Machado *et al.*, 2013; Wang *et al.*, 2014) and that the primary metabolites can be equally important for insect performance (Fernstrom, 1987; Cohen *et al.*, 1988; Waldbauer & Friedman, 1991; Thompson & Redak, 2000; Simpson & Raubenheimer, 2009; Roeder & Behmer, 2014), the question arises as to whether they could be responsible for the observed susceptibility of jasmonate-deficient plants. We investigated this potentially overlooked aspect of plant–herbivore interactions by studying the role of jasmonates in the regulation of carbohydrate accumulation in *N. attenuata* leaves, including potential underlying mechanisms, and the contribution of jasmonate-dependent carbohydrate depletion to herbivore resistance. To answer the first question, we measured sugar concentrations and invertase activity in *N. attenuata* genotypes that are impaired to different degrees in their jasmonate biosynthesis, signaling and/or perception. To answer the second question, we evaluated *M. sexta* growth when feeding on plants, artificial and semi-artificial diets with different sugar concentrations. Our results reveal that soluble sugar concentrations reduce rather than enhance jasmonate-dependent plant resistance.

## Materials and Methods

### Plant material

Transgenic inverted repeat (ir) and empty vector (EV) control (A-03-9-1) *Nicotiana attenuata* Torr. Ex. Watson plants were used in this study. The characteristics of these previously characterized different genotypes are summarized in Table 1. In addition, we produced a hemizygous cross between inverted repeat allene oxide cyclase (irAOC; line A-07-457-1) and inverted repeat ribulose-1,5-bisphosphate carboxylase/oxygenase activase (irRCA; line A-03-462-7-1) lines by removing anthers from flowers of irRCA plants before pollen maturation and pollinating the stigmas with pollen from irAOC plants.

### Planting conditions

Before planting, all seeds were surface sterilized and germinated on Gamborg's B5 medium (Krügel *et al.*, 2002). Ten-day old seedlings were transferred to Teku pots for another 10 d (Pöppelmann GmbH & Co. KG, Lohne, Germany) before planting them into 1-l pots filled with washed sand or standard substrate. Plants were grown at 45–55% relative humidity and 24–26°C during days and 23–25°C during nights under 16 h of light (06:00–22:00 h). Plants were watered twice every day.

### Soluble sugar, starch and protein concentrations in jasmonate signaling-impaired lines across different developmental stages

To investigate the possible role of jasmonates in the regulation of primary metabolism in *N. attenuata*, we measured glucose, fructose, sucrose, starch and soluble protein concentrations in the rosette leaves of jasmonate biosynthesis-deficient irAOC and jasmonate perception-impaired inverted repeat coronatine insensitive 1 (irCOI1) plants. As endogenous jasmonate levels change

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

| Genotype | Gene silenced | Impaired function | Phenotype | Reference |
|---|---|---|---|---|
| irSIPK | Salicylic acid-induced protein kinase | Early jasmonate signaling | Reduced levels of jasmonates | Meldau *et al.* (2009) |
| irWIPK | Wound-induced protein kinase | | | |
| irGLA1 | Glycerolipase A1 | Jasmonate biosynthesis | | Bonaventure *et al.* (2011) |
| irAOS | Allene oxide synthase | | | Kallenbach *et al.* (2012) |
| irAOC | Allene oxide cyclase | | | |
| irOPR3 | 12-oxo-phytodienoic acid reductase | | | |
| irJAR4/6 | JA-Ile synthetase | | Reduced levels of JA-Ile | Wang *et al.* (2008) |
| irCOI1 | Coronatine-insensitive 1 | JA-Ile perception | Reduced JA-Ile perception | Paschold *et al.* (2007) |
| irRCA | Ribulose-1,5-bisphosphate carboxylase/oxygenase activase | Photosynthesis | Reduced photosynthetic activity | Mitra & Baldwin (2008) |
| irAOC × irRCA | Allene oxide cyclase and ribulose-1,5-bisphosphate carboxylase/oxygenase activase | Jasmonate biosynthesis and sugar metabolism | Reduced sugar concentrations compared with irAOC plants | Present study |

JA-Ile, jasmonoyl-L-isoleucine.

over plant development (Abdala *et al.*, 2002), we measured sugar concentrations at four different developmental stages: early rosette (32 d after germination; DAG), rosette (38 DAG), elongation (44 DAG) and early flowering (50 DAG). Sugar and starch concentrations were quantified as described by Machado *et al.* (2013). Briefly, soluble sugars were extracted from plant tissue using 80% (v/v) ethanol, followed by an incubation step (20 min at 80°C). Pellets were re-extracted twice with 50% (v/v) ethanol (20 min at 80°C). Supernatants from all extraction steps were pooled together, and sucrose, glucose and fructose were quantified enzymatically as described by Velterop & Vos (2001). The remaining pellets were used for an enzymatic determination of starch (Smith & Zeeman, 2006). In addition, total soluble protein was quantified (Bradford, 1976). As protein solubility is affected by pH, total soluble protein levels may be underestimated by this method. Five independent replicates of each genotype and developmental stage were analyzed. Plant leaves were harvested at 13:00 h and flash frozen in liquid nitrogen for analysis.

### Constitutive jasmonate and soluble sugar concentrations in jasmonate-deficient plants

To assess the importance of jasmonates for sugar accumulation, we evaluated eight different genetically engineered lines that differ in their capacity to produce jasmonates because they are deficient in either jasmonate biosynthesis or in the upstream signaling network. We measured glucose, fructose and sucrose concentrations, as well as constitutive JA and jasmonoyl-L-isoleucine (JA-Ile), in the leaves of rosette stage plants of all genotypes. Phytohormone measurements were carried out as described by Machado *et al.* (2013). Plants were harvested at 10:00 h ($n = 5$). Sugars were then correlated with phytohormone levels.

### Diurnal changes in invertase activity and soluble sugar concentrations in jasmonate-deficient irAOC and EV plants

Invertases cleave sucrose into glucose and fructose following a diurnal pattern (Sturm & Tang, 1999; Nägele *et al.*, 2010). Higher invertase activity might therefore lead to higher glucose and fructose pools. To investigate whether the higher glucose and fructose concentrations observed in jasmonate biosynthesis-deficient irAOC plants can be attributed to higher invertase activity, we measured the activity of soluble and insoluble invertases and correlated the ratio of sucrose (precursor) to glucose and fructose (products) with the measured enzyme activities. Invertase activities and sugar concentrations were measured from leaf extracts of rosette stage EV and irAOC plants at five times of the day: 07:00, 10:00, 13:00, 17:00 and 21:00 h. Five independent replicates (plants) of each genotype were harvested per time point. Enzyme activities (Ferrieri *et al.*, 2013) and sugar concentrations were measured as described by Machado *et al.* (2013).

### Effect of soluble sugars on caterpillar growth

Low secondary metabolite levels are generally assumed to be responsible for the increased larval growth of herbivores on jasmonate signaling-impaired plants (Halitschke & Baldwin, 2003; Rayapuram & Baldwin, 2006; Paschold *et al.*, 2007). To determine whether the higher soluble sugar concentrations in jasmonate-deficient plants contribute to the increased *M. sexta* larval growth, we manipulated sugar concentrations *in planta* and *in vitro* and measured caterpillar growth in five different experiments as follows.

**Caterpillar growth on sugar-restored, jasmonate biosynthesis-deficient plants** To decrease soluble sugars in irAOC plants, we produced a hemizygous irAOC × irRCA line by crossing an irAOC line with an irRCA line. Silencing RCA slightly impairs photosynthetic activity in *N. attenuata* (Mitra & Baldwin, 2008), and we therefore hypothesized that a reduction in the photosynthetic capacity should reduce sugar concentrations and, consequently, the hemizygous jasmonate-deficient plants should have restored wild-type (WT) sugar concentrations. To test the validity of this assumption, sugar concentrations were measured in the leaves of EV, irRCA, irAOC and irAOC × irRCA plants at 05:00 h (end of the dark period) and 13:00 h (middle of the light period). As *M. sexta* herbivory has been shown to reduce sugar

concentrations in *N. attenuata* (Machado *et al.*, 2013), we also measured sugar concentrations after simulated (wounding and *M. sexta* oral secretion treatments, W + OS) and actual (three neonates per plant for 6 d) *M. sexta* herbivory. Intact plants served as controls ($n = 5$). *Manduca sexta* herbivory (W + OS) was simulated by rolling a fabric pattern wheel three times on each side of the midvein of fully developed rosette leaves. The wounds were immediately treated with 20 µl of a 1 : 5 (v/v) milliQ water-diluted *M. sexta* oral secretion solution. The treatments were repeated three times every other day. Following the validation of this *in vivo* approach, we determined caterpillar growth on the different genotypes. Two *M. sexta* neonates were placed on rosette stage plants and allowed to feed freely ($n = 25$). Seven and 9 d later, their mass was determined using a microbalance (Sartorius TE214S; Data Weighing Systems Inc., Elk Grove, IL, USA). *Manduca sexta* eggs were derived from an in-house colony and reared as described by Grosse-Wilde *et al.* (2011).

**Caterpillar growth on plants with reduced photosynthetically active radiation (PAR)** As an alternative means of reducing soluble sugars in irAOC plants, we reduced the amount of PAR by covering rosette leaves with a green filter (Roscolux #4430; Rosco Laboratories Inc., Stamford, CT, USA). We hypothesized that reducing PAR supply should reduce sugar concentrations in the leaves. Plants covered with a clear filter (Roscolux #000; Rosco Laboratories Inc.) were used as controls. Sugar concentrations were quantified 3 d after the start of the PAR reduction treatment as described by Machado *et al.* (2013). Plants for the sugar measurements were harvested at 09:00 h. Head space temperature and humidity, red to far-red ratios, starch (Machado *et al.*, 2013), soluble proteins (Bradford, 1976), average internode length and number of flowers were also quantified to assess whether the filters elicited shade avoidance responses and other secondary effects. To evaluate caterpillar growth, two *M. sexta* neonates were placed on rosette stage plants ($n = 30$) and allowed to feed freely. Seven and 9 d later, larval mass was determined as described earlier.

**Caterpillar growth on semi-artificial diets** *Manduca sexta*-induced jasmonate signaling depletes soluble sugars and induces secondary defensive metabolites in the leaves of *N. attenuata* plants; in contrast with EV plants, sugars are not depleted and secondary defensive metabolites are not induced in response to *M. sexta* simulated herbivory in jasmonate biosynthesis-deficient irAOC plants (Machado *et al.*, 2013). To understand whether the better *M. sexta* growth on jasmonate-deficient plants is a result of their increased sugar concentration and/or their decreased levels of secondary metabolites, we performed an experiment with semi-artificial diets in which sugars were complemented to match those of WT and control plants. The diets were prepared as described later, but the wheat germ was replaced with 25 g of dried *N. attenuata* leaves. To generate the necessary plant material, we treated irAOC and EV plants with *M. sexta* oral secretions (W + OS induction) as described by Machado *et al.* (2013). These treatments induce plant defenses and deplete sugars in the leaves of *N. attenuata* in a JA-dependent manner (Machado *et al.*, 2013). After the treatments, we collected and dried the leaves (24 h at 50°C). Plants were harvested at 13:00 h. Diets prepared with untreated plant material served as controls. Sugar concentrations in the semi-artificial diets were determined, and subsets of diet cubes were complemented with pure sugars to match WT and control levels. *Manduca sexta* growth on the different diets was then measured over 12 d. Forty-four neonates per diet type (four larvae per plate; 11 plates per diet type) were fed *ad libitum* and the diet cubes were replaced every other day. In addition, caterpillar survivorship was recorded. As sugar complementation of plants may induce secondary responses (Rolland *et al.*, 2006), the above approach allowed us to test the direct contribution of soluble sugars to herbivore growth in a plant matrix.

**Caterpillar growth on artificial diets enriched in glucose and fructose** To evaluate the individual effect of glucose and fructose on *M. sexta* growth, we prepared artificial diets with different concentrations of glucose and/or fructose (see later, Fig. 7, for treatment combinations) and measured *M. sexta* growth. The diets were prepared essentially as described by Pohlon & Baldwin (2001) without sucrose, plant material and antibiotics. Briefly, 17 g of agar were dissolved in 500 ml of water at 50°C and mixed with 55 g wheat germ, 12 g yeast extract, 9 g Wesson salt mixture, 3.5 g ascorbic acid, 2.5 g cholesterol, 1.5 g sorbic acid, 5 ml raw linseed oil, 1.5 ml formalin and 9 ml vitamin mixture (100 mg nicotinic acid, 500 mg riboflavin, 233.5 mg thiamine, 233.5 mg pyridoxine, 233.5 mg folic acid and 20 mg $l^{-1}$ biotin in water). The produced food was aliquoted into small plastic boxes and kept at 8°C until use. Glucose and fructose were dissolved in water and added to the diet cubes. Diets were freshly prepared and replaced every other day. Forty caterpillars (four larvae per plate; 10 plates per diet type) were fed *ad libitum* in a climate chamber (45–55% relative humidity, 24–26°C during days and 23–25°C during nights under 16 h of light). Larval mass was determined as described earlier, 7 and 9 d after the beginning of the experiment ($n = 40$).

**Interaction between protein and soluble sugars** The performance of insect herbivores depends, among other factors, on protein : carbohydrate ratios (Raubenheimer *et al.*, 2005; Simpson & Raubenheimer, 2009; Roeder & Behmer, 2014). To investigate whether the observed negative effect of increased dietary soluble sugars on *M. sexta* growth changes with the amount of available protein, we prepared artificial diets with variations in sugar and protein concentration and measured *M. sexta* larval mass and the amount of ingested diet, and calculated the efficiency of conversion of ingested food (Waldbauer, 1968). Diets were prepared according to Pohlon & Baldwin (2001). Sucrose was replaced by increasing concentrations of glucose and fructose (1, 6 and 12 mg $g^{-1}$ of diet) to mimic the actual differences in soluble sugar profiles between jasmonate biosynthesis-deficient irAOC and WT plants. To increase the protein concentration of the diets, casein was added at concentrations of 50 or 150 mg $g^{-1}$ FW. Soluble protein concentrations in plants estimated by the Bradford method can reach 14.47 mg $g^{-1}$ in the leaves of some plant species (Ruiz & Romero, 1999). Thirty neonates (three

larvae per plate; 10 plates per diet type) were fed *ad libitum* and the above parameters were determined 9 d after the beginning of the experiment ($n = 30$).

### Statistics

Unless otherwise stated, statistical tests were carried out with Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA) using analysis of variance. Levene's and Shapiro–Wilk tests were applied to determine error variance and normality. Holm–Sidak and Dunn's *post hoc* tests were used for pairwise or multiple comparisons. Datasets from experiments that did not fulfill the assumptions for ANOVA were natural log-, root square- or rank-transformed before analysis. The effect of semi-artificial diets on caterpillar survivorship was analyzed in R (R Development Core Team, 2012) using generalized linear models (GLMs), under a quasibinomial distribution with *F*-test. Residual analysis was carried out to verify the suitability of error distribution and model fitting. Details on specific tests carried out in each experiment are provided in Supporting Information Notes S1.

## Results

### Jasmonate signaling negatively affects leaf glucose and fructose concentrations

Leaf soluble protein levels of jasmonate perception-impaired irCOI1 and jasmonate biosynthesis-deficient irAOC plants were similar to those observed in jasmonate-competent EV plants across different developmental stages (Fig. 1a–d). Starch did not differ between genotypes at early rosette (Fig. 1e), rosette (Fig. 1f) and elongated (Fig. 1g) stages. Early flowering irCOI1, but not irAOC, plants contained higher leaf starch concentrations than EV plants (Fig. 1h). Glucose and fructose concentrations were higher in jasmonate signaling-impaired plants compared with EV controls at early rosette (Fig. 1i), elongated (Fig. 1k) and early flowering (Fig. 1l) stages. At the rosette stage, only fructose concentrations in irAOC plants were elevated compared with EV controls (Fig. 1j). Sucrose concentrations did not differ between genotypes at any of the evaluated developmental stages (Fig. 1i–l).

### JA concentrations are negatively correlated with leaf soluble sugars across different jasmonate-deficient genotypes

Across eight jasmonate-deficient transgenic lines (Fig. 2a), we found significant variations in soluble sugar, JA and JA-Ile concentrations (Fig. 2b,c). A significant negative correlation between soluble sugar and constitutive JA concentrations ($P < 0.001$) was observed (Fig. 2b). By contrast, no significant correlation between soluble sugar and JA-Ile concentrations ($P = 0.213$) was found (Fig. 2c). Together, the above experiments demonstrate that jasmonates negatively affect soluble sugar concentrations in *N. attenuata* leaves.

### Jasmonate-dependent sugar suppression is correlated with decreased invertase activity

Over the course of the day, we found that leaves of jasmonate biosynthesis-deficient irAOC plants accumulated less sucrose from 13:00 to 17:00 h, but more glucose and fructose from 10:00 to 21:00 h, compared with EV controls (Fig. 3a,b). Moreover, soluble invertase activity was increased in jasmonate biosynthesis-deficient irAOC plants compared with EV plants from 13:00 to 21:00 h (Fig. 3c), an effect which correlated negatively with the ratios of sucrose to fructose and glucose across the different samples and time points (Fig. 3d). By contrast, insoluble invertase activity was not altered by jasmonates and not correlated with soluble sugar ratios (Fig. 3e,f).

### Jasmonate-dependent sugar suppression improves herbivore weight gain

To understand whether higher leaf sugar concentrations in jasmonate biosynthesis-deficient irAOC plants improve *M. sexta* growth, we reduced sugar concentrations in irAOC plants by silencing RCA activity. A reduction in RCA activity did not affect soluble sugars in EV plants, but decreased glucose and fructose concentrations in irAOC plants (Fig. 4, S1). The partial restoration of WT sugar concentrations in the irAOC × irRCA crosses was even more pronounced in herbivory-induced plants (Fig. 4). Sucrose and soluble protein concentrations remained largely unchanged (Figs 4, S2), apart from a slight increase in constitutive sucrose concentrations in irAOC × irRCA plants. From these results, we deduced that silencing RCA partially restored WT sugar concentrations in jasmonate biosynthesis-deficient irAOC plants, and these lines could be used to test the influence of soluble sugars on *M. sexta* growth *in planta*. Our initial expectation was that increased glucose and fructose concentrations would increase *M. sexta* growth. In contrast with this hypothesis, we found that the reduced sugar concentrations in irAOC × irRCA plants increased *M. sexta* growth even beyond the highly increased mass gain in the irAOC plants (Fig. 4j). This result suggests that jasmonate-dependent sugar depletion reduces rather than enhances plant resistance.

### *Manduca sexta* gains more weight on PAR-limited, sugar-deprived plants

As a second approach to manipulate plant sugar concentrations, we reduced PAR supply by 43% (Fig. 5a). As expected, reducing PAR significantly reduced sugar concentrations in both EV and irAOC plants (Fig. 5b). Although the green filters slightly changed the red : far-red ratios (Fig. S3a), we found little phenotypic evidence for the activation of shade avoidance responses in PAR-reduced plants (Fig. S4a,b). We also found no changes in head space temperature and humidity (Fig. S3b,c). Starch and soluble protein concentrations also remained unaltered (Fig. S3c,d). Overall, *M. sexta* larvae gained more weight on irAOC plants than on EV plants (Fig. 5c). PAR reduction significantly increased caterpillar weight gain independent of the

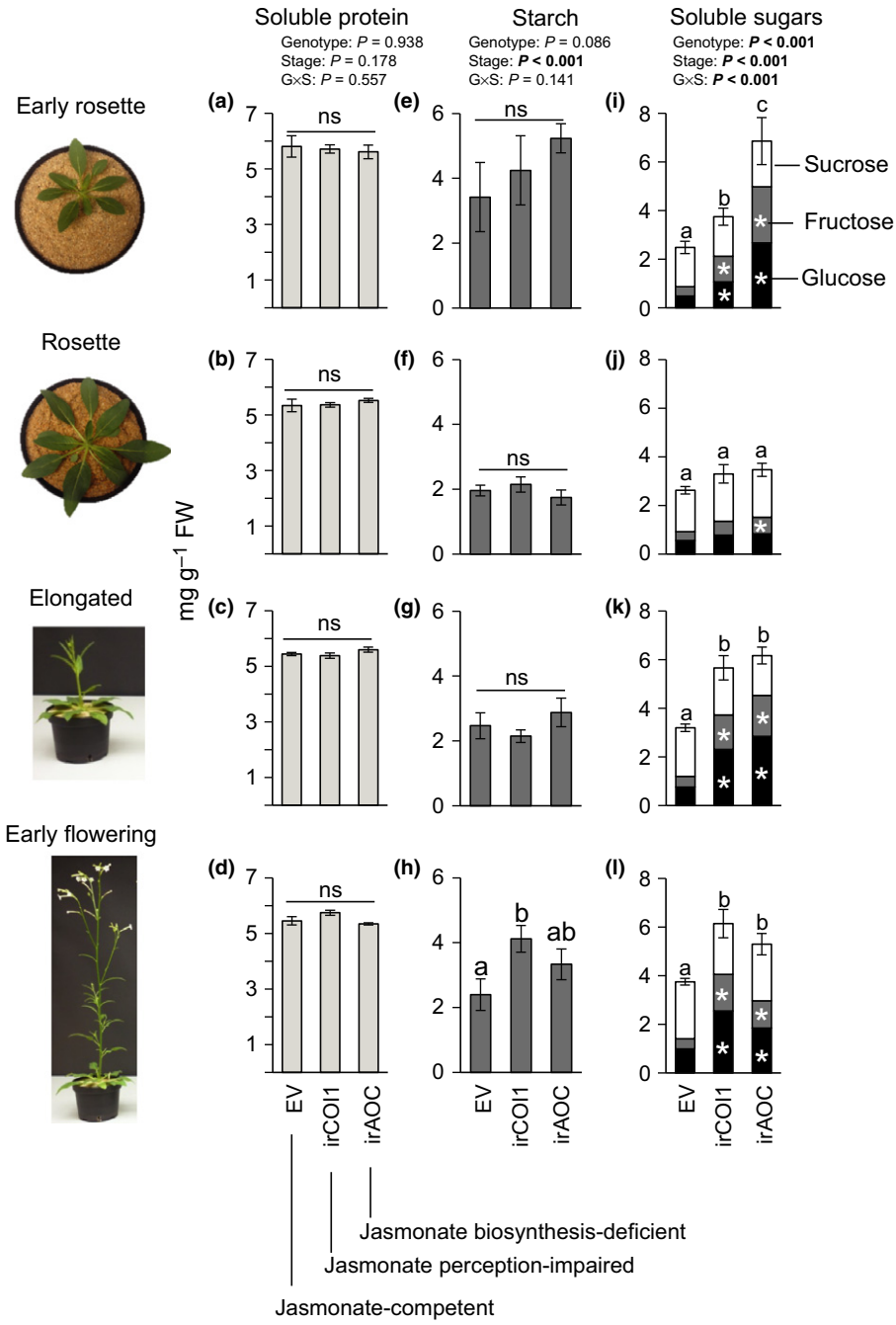


**Fig. 1** Jasmonate signaling-impaired *Nicotiana attenuata* plants accumulate higher sugar concentrations than jasmonate-competent empty vector (EV) plants across different developmental stages. Average (± SE) (a–d) soluble protein, (e–h) starch and (i–l) soluble sugar concentrations of rosette leaves at (a, e, i) early rosette, (b, f, j) rosette, (c, g, k) elongated and (d, h, l) early flowering stages. Different letters indicate significant differences ($P < 0.05$, Holm–Sidak *post hoc* tests) in total sugar (glucose, fructose and sucrose) concentration among genotypes within developmental stages. Asterisks indicate significant differences of each individual metabolite (glucose, fructose or sucrose) concentration among genotypes (irAOC or irCOI1) compared with EV within developmental stages (Holm–Sidak *post hoc* tests: *, $P < 0.05$; ns, not significant) ($n = 5$).

plant's capacity to produce jasmonates (Fig. 5c). As irAOC plants contain higher sugar levels, but also lower secondary metabolites, compared with EV plants, both factors potentially contribute to the observed caterpillar growth rates.

## *Manduca sexta* grows better on low secondary metabolite and low sugar-containing semi-artificial diets

In a third approach, we complemented semi-artificial diets with soluble sugars. This resulted in seven different diets with different sugar concentrations and added plant materials (Fig. 6a). Sugar complementation effectively increased control EV sugar concentrations to match those of control irAOC plants, and herbivory-suppressed EV sugar concentrations to match those of control EV and herbivory-induced irAOC concentrations (Fig. 6a). *Manduca sexta* weight gain did not differ between larvae that fed on uninduced EV and irAOC diets (Fig. 6b, bars A, C). When sugar concentrations in uninduced EV diets were complemented to uninduced irAOC concentrations, *M. sexta* growth was reduced (Fig. 6b, bar B). When feeding on W + OS-induced plant material, *M. sexta* growth was lower on EV than on irAOC diet (Fig. 6b, bars D, G). When sugar concentrations of W + OS-induced EV plant material were complemented to uninduced EV controls or to uninduced irAOC concentrations, *M. sexta* growth

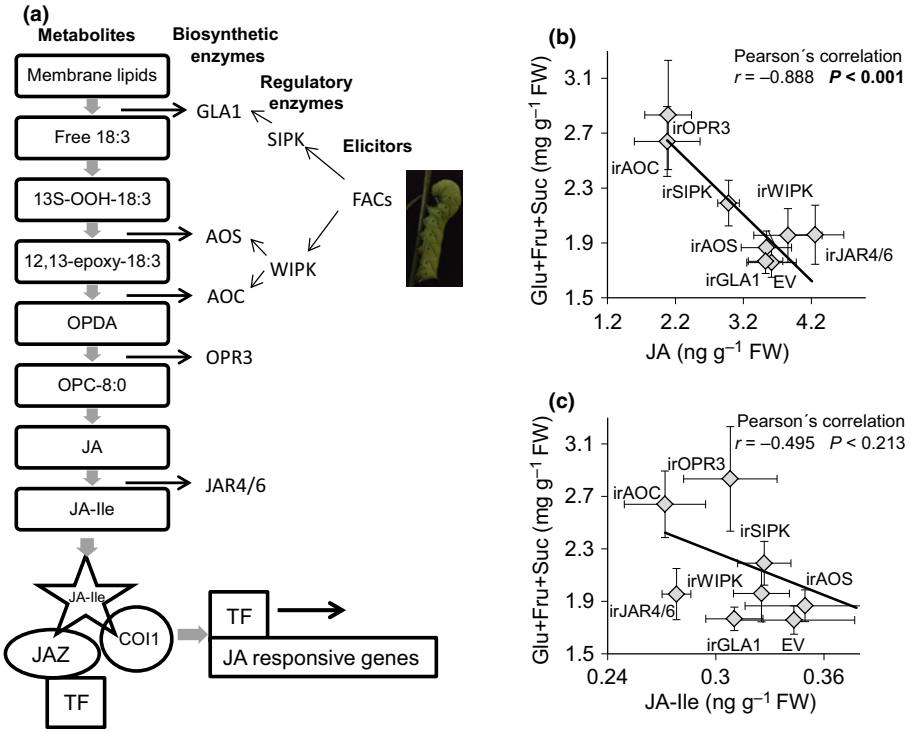


**Fig. 2** Constitutive jasmonic acid and soluble sugar concentrations are negatively correlated. (a) Schematic representation of the jasmonate signaling cascade. (b, c) Correlation between average (± SE) soluble sugars (glucose, fructose and sucrose; Glu + Fru + Suc) and (b) average (± SE) constitutive jasmonic acid levels (JA) or (c) constitutive jasmonoyl-L-isoleucine (JA-Ile) levels. GLA1, glycerolipase A1; SIPK, salicylic acid-induced protein kinase; FACs, fatty acid–amino acid conjugates; WIPK, wound-induced protein kinase; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPR3, 12-oxophytodienoic acid (OPDA) reductase; OPC-8:0, 3-oxo-2-(2′-pentenyl)-cyclopentane-1-octanoic acid; JAR 4/6, JA-amino acid synthetase; COI1, coronatine insensitive 1; JAZ, Jasmonate ZIM-domain protein; TF, transcription factors. Metabolite analysis was carried out in five independent replicates of each *Nicotiana attenuata* genotype.

was further reduced (Fig. 6b, bars E, F). A similar pattern was observed for caterpillar survival (Fig. 6c). Taken together, these results show that the W + OS-triggered induction of defense reduces *M. sexta* growth and survival in a jasmonate-dependent manner, but that the jasmonate-dependent W + OS-induced sugar depletion increases *M. sexta* growth and thereby compromises induced resistance.

### *Manduca sexta* grows better on low sugar-containing artificial diets

To disentangle the individual contribution of glucose and fructose to *M. sexta* growth suppression, we complemented artificial diets with different combinations of the two sugars at physiologically relevant concentrations. *Manduca sexta* growth strongly decreased with increasing amounts of glucose and fructose in a dose-dependent manner, independent of the combination of sugars (Fig. 7). On an individual basis, increased fructose decreased *M. sexta* growth to a greater extent than did increased glucose (Fig. 7).

### Excess protein reverses the negative effect of soluble sugars on *M. sexta* weight gain

At a protein supply of 50 mg $g^{-1}$ FW, *M. sexta* grew less well on higher soluble sugar diets in a dose-dependent manner (Fig. 8a). However, when excess protein at a concentration of 150 mg $g^{-1}$ FW was offered, the opposite effect was observed: *M. sexta* now gained more weight on sugar-rich diets (Fig. 8a). We also found that the amount of ingested diet decreased with increasing sugar concentrations in a protein-independent manner (Fig. 8b). The efficiency of conversion of ingested food did not change under normal protein supply, but tended to increase with sugar concentrations under excess protein (Fig. 8c). These results demonstrate that the negative effect of soluble sugars on *M. sexta* growth depends on the protein content of the food source. Under natural conditions, the amount of available protein in the leaves would result in a negative effect of sugars on *M. sexta* growth, as protein concentrations in plant leaves are below 50 mg $g^{-1}$ FW.

## Discussion

Our experiments demonstrate that jasmonates inhibit soluble invertases and reduce glucose and fructose concentrations in *N. attenuata* leaves, and that this reduction directly compromises plant resistance by increasing *M. sexta* growth and survival.

Across different developmental stages, times of day, and transgenic events, jasmonate signaling negatively influenced the concentrations of glucose and fructose in *N. attenuata* leaves, whereas only minor changes in starch and sucrose were found. This suggests that jasmonates specifically influence soluble monosaccharide concentrations. The differences between WT and jasmonate signaling-impaired plants were weaker at the late rosette stage than in younger and older plants. It remains to be determined to what extent this variation is a result of variation in jasmonate biosynthesis or downstream signaling. Constitutive and induced jasmonate levels are reduced in flowering *N. attenuata* plants, an effect that is correlated with the lower expression of jasmonate-dependent defenses (van Dam *et al.*, 2001; Kaur *et al.*, 2010; Diezel *et al.*, 2011; Onkokesung *et al.*, 2012). The fact that jasmonate-dependent sugar regulation did not follow this pattern

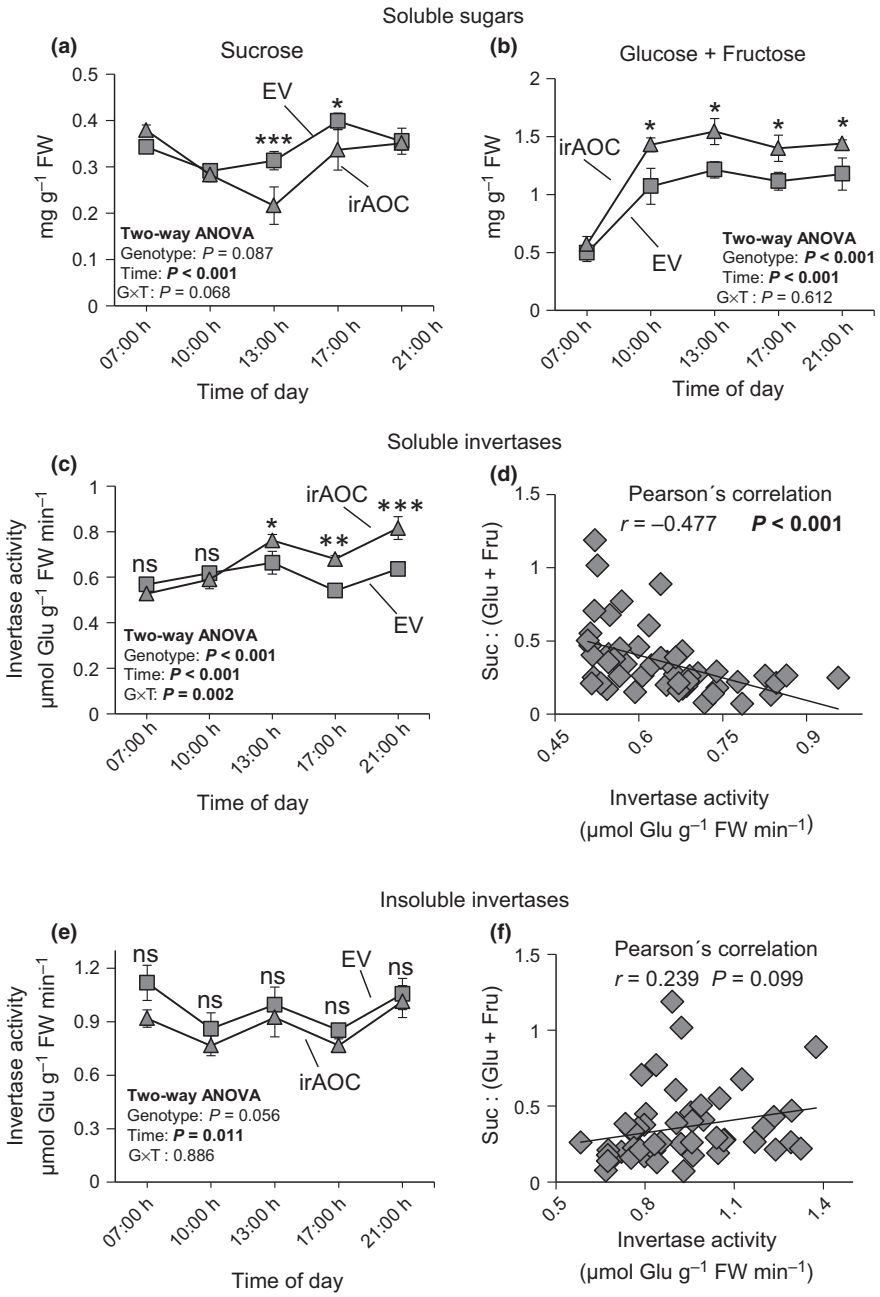


**Fig. 3** Diurnal jasmonate-dependent suppression of soluble sugars is associated with decreased invertase activity. Average (± SE) (a) sucrose and (b) glucose and fructose concentrations. Average (± SE) (c) soluble and (e) insoluble invertase activity. (d, f) Correlation between the ratio of sucrose (Suc) to glucose (Glu) and fructose (Fru) and (d) soluble invertase activity or (f) insoluble invertase activity. Asterisks indicate significant differences within each time point (Holm–Sidak *post hoc* tests: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant). Metabolite and enzyme activity measurements were carried out in five independent replicates (individual *Nicotiana attenuata* plants) for each genotype and time point. EV, empty vector; irAOC, allene oxide cyclase-silenced plants.

suggests a role of downstream signaling, rather than a direct effect of jasmonate biosynthesis, on the observed developmental patterns.

We found that the total soluble protein remained constant in the leaves of *N. attenuata* plants across different developmental stages, times of day, transgenic events and plant treatments. It is important to note that we measured protein concentration by the Bradford method. As this method requires acidic conditions, and as the solubility of RuBisCO, one of the most abundant proteins, is decreased under low pH, we may have underestimated the total protein concentrations. However, using $^{15}N$ labeling and LC-$MS^E$, it was demonstrated that investment into RuBisCO biosynthesis is not changed in jasmonate-deficient inverted repeat lipoxygenase (irLOX3) *N. attenuata* plants (Ullmann-Zeunert *et al.*, 2013). However, herbivore attack decreased RuBisCO levels in WT and, albeit to a lesser extent, irLOX3 plants (Ullmann-Zeunert *et al.*, 2013). A detailed analysis of the most abundant soluble proteins in jasmonate signaling-impaired plants using similar approaches might help us to understand whether and how jasmonate signaling regulates soluble protein levels in plants in more detail.

Over the course of the day, we observed that deficiencies in jasmonate signaling increased glucose and fructose concentrations, as well as invertase activity, in the leaves of rosette stage *N. attenuata* plants during the light phase. We also noted a slight suppression in sucrose concentrations around midday. Based on

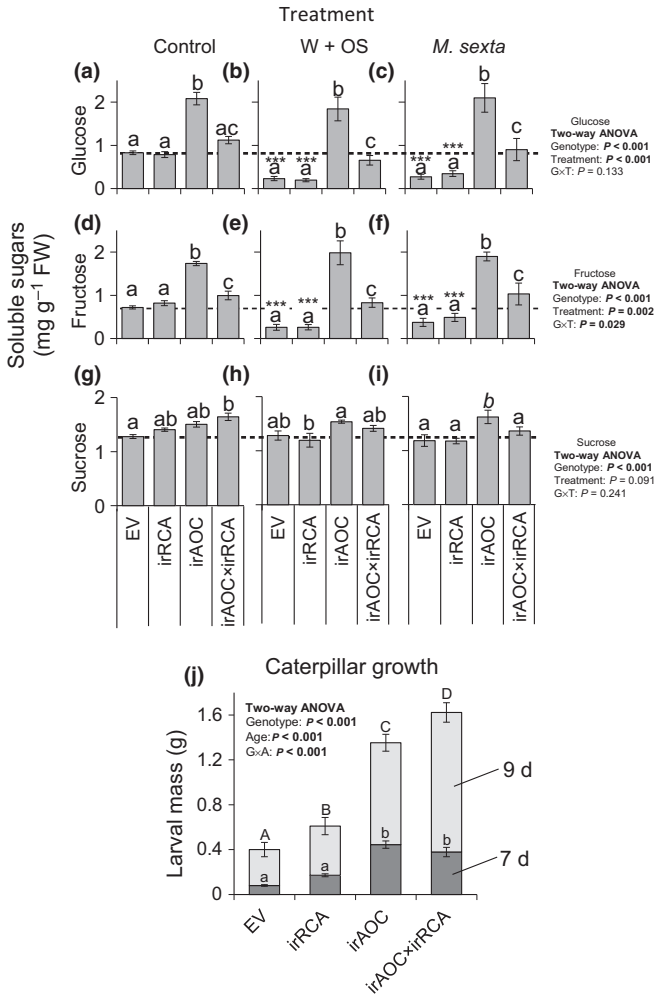


**Fig. 4** Reducing soluble sugar concentrations through ribulose-1,5-bisphosphate carboxylase/oxygenase activase (RCA) inhibition increases *Manduca sexta* growth. Average (± SE) (a, b, c) glucose, (d, e, f) fructose and (g, h, i) sucrose concentrations of (a, d, g) control, (b, e, h) wounding and *M. sexta* oral secretion (W + OS)-treated and (c, f, i) *M. sexta*-attacked plants harvested at 13:00 h. (j) Average (± SE) mass of *M. sexta* larvae reared on different *Nicotiana attenuata* genotypes ($n = 35$–$50$). irRCA, ribulose-1,5-bisphosphate carboxylase/oxygenase activase-silenced plants; irAOC, allene oxide cyclase-silenced plants; irRCA × irRCA, hemizygous crosses between AOC- and RCA-silenced plants; EV, empty vector-transformed plants. Different letters indicate significant differences (Holm–Sidak *post hoc* tests: $P < 0.05$) among genotypes within each treatment and metabolite for (a–i) and within time point for (j). Asterisks indicate significant differences among each treatment and control plants within each genotype and metabolite (Holm–Sidak *post hoc* tests: ***, $P < 0.001$). Dashed lines indicate the levels of each metabolite for intact EV plants. Metabolite measurements were carried out in five independent replicates of each genotype and treatment. (j) The lower portion represents the weight that caterpillars reached after 7 d, and the total height of the bars represents the weight that caterpillars reached after 9 d. Therefore, the upper portion represents only the weight that caterpillars gained between day 7 and 9.

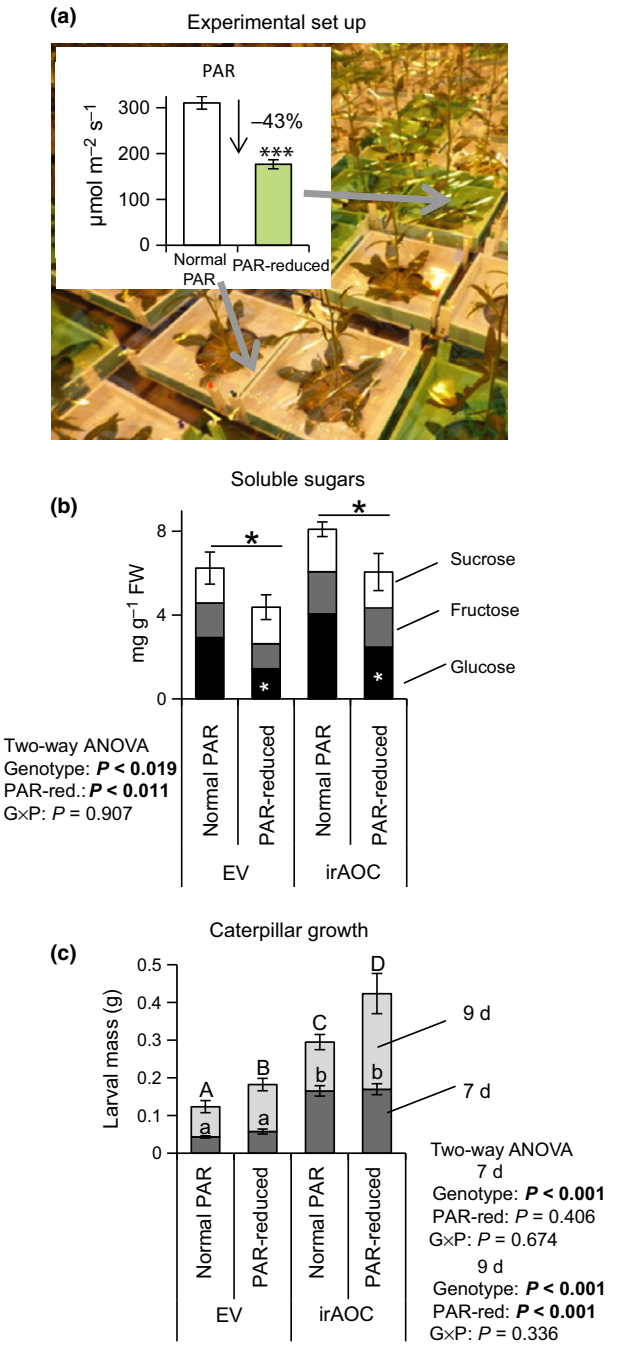


**Fig. 5** Reduction in photosynthetically active radiation (PAR) in *Nicotiana attenuata* plants decreases soluble sugars and increases *Manduca sexta* growth in a jasmonate-independent manner. (a) PAR under PAR-reduced and normal glasshouse PAR conditions. (b) Average (± SE) sugar concentrations of *N. attenuata* plants under PAR-reduced and normal conditions. (c) Average (± SE) mass of 7- and 9-d-old *M. sexta* larvae feeding on plants grown under PAR-reduced and normal conditions ($n = 36$–$48$). Asterisks in (a) indicate a significant effect of filter treatments on PAR (Holm–Sidak *post hoc* tests: ***, $P < 0.001$). Asterisks in (b) indicate significant differences in sugar concentration between plants under PAR-reduced and normal PAR conditions within each genotype (Holm–Sidak *post hoc* tests: *, $P < 0.05$). Different letters indicate significant differences (Holm–Sidak *post hoc* tests: $P < 0.05$) among treatments within caterpillar age. Metabolite measurements were carried out in five independent replicates of each genotype and treatment. EV, empty vector; irAOC, allene oxide cyclase-silenced plants.

the positive correlation between invertase activities and precursor to product ratios of the different sugars, we propose that jasmonates might regulate sugar concentrations through the suppression of invertase activity. Invertases are well known to control the

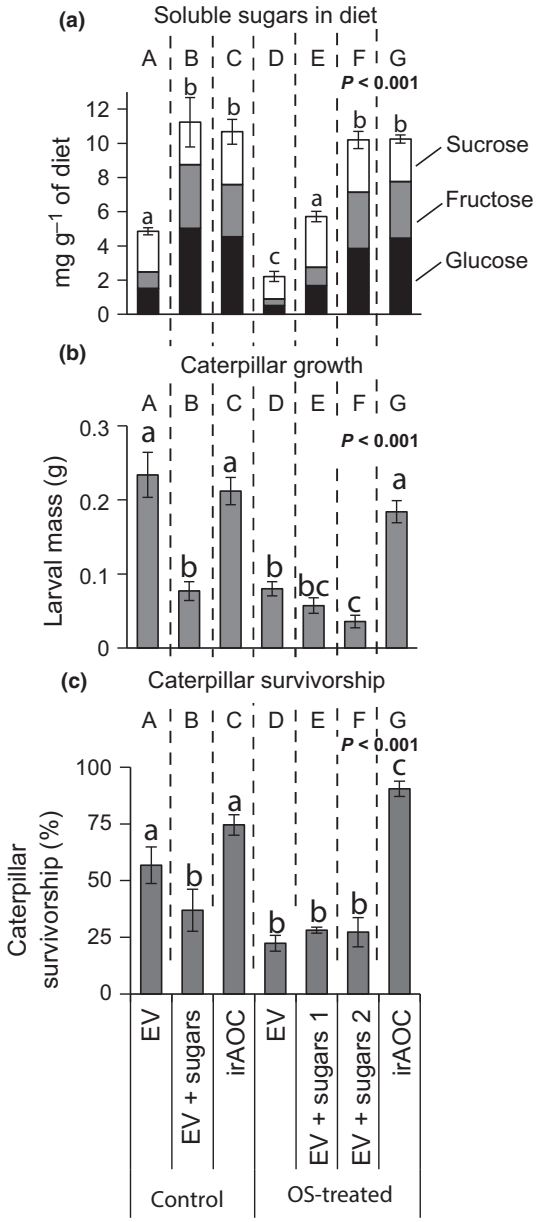


**Fig. 6** Soluble sugar complementation of a semi-artificial diet reduces *Manduca sexta* growth and survival. (a) Average (± SE) soluble sugars in diets. (b) Average (± SE) mass of *M. sexta* larvae reared on semi-artificial diets that differ in their primary and secondary chemical profiles ($n = 10$–38). (c) Average (± SE) proportion of living caterpillars at the end vs the beginning of the experiment. Different letters in (a) and (b) indicate significant differences in total sugar and caterpillar growth (Dunn's *post hoc* tests: $P < 0.05$). Different letters in (c) indicate significant differences in caterpillar survival (*F*-test: $P < 0.05$). Uppercase letters (A–G) serve as a guide for the comparisons. EV, empty vector; irAOC, allene oxide cyclase-silenced plants.

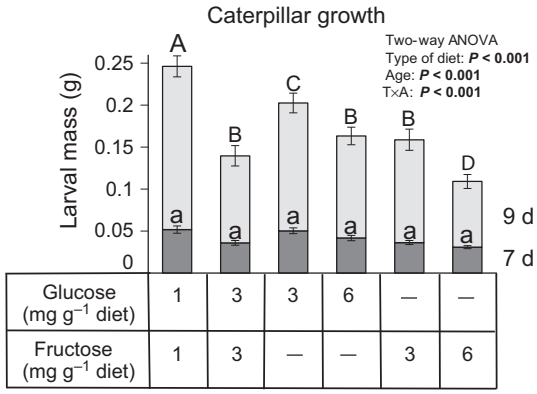


**Fig. 7** *Manduca sexta* grow less well on artificial diets containing higher glucose and fructose concentrations. Average (± SE) mass of 7- and 9-d-old *M. sexta* larvae reared on artificial diets containing variable amounts of glucose and fructose ($n = 32$–39). Different letters indicate significant differences within a time point (Holm–Sidak *post hoc* tests: $P < 0.05$).

ratio of glucose and fructose to sucrose (Zrenner *et al.*, 1996; Ohyama & Hirai, 1999; Tang *et al.*, 1999; Jin *et al.*, 2009; Bhaskar *et al.*, 2010). Silencing of a vacuolar invertase gene in potato, *Solanum tuberosum*, was found to decrease vacuolar invertase activity, increase sucrose and reduce glucose and fructose (Bhaskar *et al.*, 2010). Similarly, Zrenner *et al.* (1996) found a positive correlation between the ratio of hexoses to sucrose and acid-soluble invertase activity in different potato cultivars. Silencing of a soluble acid invertase resulted in lower ratios of hexoses to sucrose. It is noteworthy to mention that other studies in poplar and thale cress have documented that exogenous jasmonate application increases invertase activity (Arnold & Schultz, 2002; Bogatek *et al.*, 2002; Arnold *et al.*, 2004; Ferrieri *et al.*, 2013; Horibe *et al.*, 2013). The fact that constitutive jasmonate deficiency led to higher invertase activity in *N. attenuata* leaves suggests that the outcome of induced jasmonates on invertase activity might be determined by their endogenous concentrations.

Although alteration in invertase activity can change the ratio of glucose and fructose to sucrose, the increase in glucose and fructose is not always proportional to the decrease in sucrose concentration (Tang *et al.*, 1999; Bhaskar *et al.*, 2010), indicating that total sugar pools might also be regulated by other factors, including, for example, changes in photosynthetic efficiency, carbon assimilation, glycolytic activity, sucrose synthase activity, sucrose transporter activities and mechanisms of phloem loading/unloading. Indeed, reducing the activity of RCA, which modulates the activity of RuBisCO, the enzyme that carries out the first major step of $CO_2$ fixation in plants (Raines, 2003), in jasmonate biosynthesis-deficient irAOC plants reduced glucose and fructose concentrations by 54% and 57%, respectively, suggesting that jasmonate deficiency might affect sugar accumulation via the regulation of this enzyme. Although we have no evidence for changes in glycolytic enzyme activity, sucrose synthase activity, sucrose transporter activities and mechanisms of phloem loading/unloading, future studies might investigate their contribution to the higher accumulation of glucose and fructose in jasmonate signaling-impaired plants.

Given the importance of jasmonates for plant–herbivore interactions, we were interested in understanding whether and how the jasmonate-dependent reduction of soluble sugars influences the resistance of *N. attenuata* to *M. sexta* caterpillars. Through four orthogonal lines of evidence, we show that *M. sexta* growth is reduced, rather than enhanced, through increased dietary

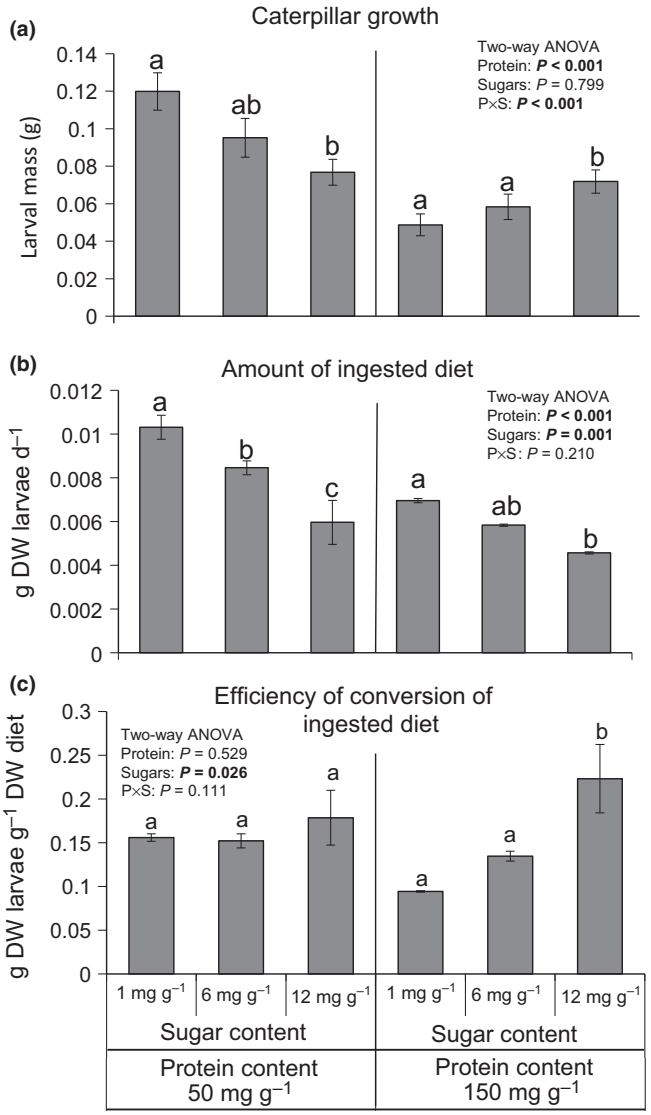


**Fig. 8** Increasing protein supply beyond natural concentrations inverts the effect of sugars on *Manduca sexta* growth. (a) Average (± SE) mass of *M. sexta* larvae reared on artificial diets containing different amounts of protein and soluble sugars ($n = 23$–30). (b) Average (± SE) amount of ingested diets by *M. sexta* larvae. (c) Average (± SE) efficiency of conversion of ingested food. Different letters indicate significant differences (Holm–Sidak *post hoc* tests: $P < 0.05$) within type of diet.

carbohydrates, an effect that is associated with a reduction in the amount of ingested food. First, reducing sugar concentrations through RCA silencing in jasmonate-deficient plants significantly improved *M. sexta* growth. irAOC plants are almost completely deficient in many defensive secondary metabolites (Machado *et al.*, 2013; Fragoso *et al.*, 2014). Therefore, this result points to a secondary metabolite-independent growth effect of soluble sugars on *M. sexta*. The better growth of *M. sexta* on nonhemizygous irRCA plants has been attributed to the reduced levels of secondary metabolites, an effect driven by the RCA-mediated redirection of the bioactive oxylipin JA-Ile to the inactive methyl jasmonate (Mitra & Baldwin, 2008, 2014). However, it is possible that this effect is also the result of reduced sugar concentrations in this line at certain times of the day (see Supporting Information, Fig. S1). Second, reducing sugars by limiting PAR supply also improved *M. sexta* growth independent of the plant's capacity to produce jasmonates. Although PAR reduction may have additional effects on plant physiology, we did not find any evidence for either the shade avoidance response or changes in starch and protein production in plants growing under PAR-reduced conditions. Furthermore, the fact that we observed similar PAR effects on caterpillar growth in EV and irAOC plants allows us to exclude secondary effects on plant secondary metabolites as key determinants of insect growth (Paschold *et al.*, 2007). Third, supplementing minimal artificial diets containing dried plant material with sugars reduced *M. sexta* growth and survival. Artificial diets containing plant material have been used in previous studies to assess the contribution of jasmonate-induced changes in plant metabolism to *M. sexta* growth (Pohlon & Baldwin, 2001). *Manduca sexta* grows less well on artificial diets containing jasmonate-treated *N. attenuata* plant material than on diets containing nontreated plant material, an effect that is positively correlated with the jasmonate-dependent induction of protease inhibitors (PIs) and nicotine (Pohlon & Baldwin, 2001). The fact that caterpillars grew less well on induced EV than irAOC plants confirms that this assay can be used to reproduce natural resistance patterns, whilst allowing for the complementation of semi-artificial diet with soluble sugars without the confounding effect of sugar signaling on plant physiology. Fourth, complementing artificial diets with physiologically relevant concentrations of individual soluble sugars confirmed their negative, dose-dependent effect on *M. sexta* growth at physiologically relevant protein concentrations.

Carbohydrate-rich artificial diets have been shown to reduce insect performance (Raubenheimer & Simpson, 1997; Lee *et al.*, 2003; Raubenheimer *et al.*, 2005; Babic *et al.*, 2008; Merkx-Jacques *et al.*, 2008) and survival (Raubenheimer *et al.*, 2005) in earlier studies, effects that are associated with a greater propensity to store the excess of ingested carbohydrate as body fat (Chippindale *et al.*, 1996; Simpson *et al.*, 2004; Warbrick-Smith *et al.*, 2006). Although we did not determine lipid concentrations in the caterpillars, the fact that the semi-artificial diet experiment showed that larval survival and weight gain were positively correlated indicates that the heavier caterpillars are not necessarily fatter, but also fitter. Direct measurements of body fat would be necessary to confirm this hypothesis. The post-ingestion mechanisms that insects use to cope with an excess of dietary carbohydrates include the down-regulation of carbohydrate-catabolizing enzymes (Kotkar *et al.*, 2009; Clissold *et al.*, 2010), the increase in respiration rates (Zanotto *et al.*, 1997), the up-regulation of glucose-oxidizing enzymes (Merkx-Jacques & Bede, 2005) and the increase in carbohydrate egestion (Telang *et al.*, 2003; Babic *et al.*, 2008). In addition to the storage of excess dietary carbohydrates as body fat, the earlier mentioned mechanisms might result in metabolic costs for caterpillars that potentially reduce their optimal growth and development.

Insect guts host an enormous and phylogenetically diverse group of microorganisms (Engel & Moran, 2013). Although their beneficial roles are increasingly being recognized (Salem

*et al.*, 2013, 2014), they are also potentially deleterious (Basset *et al.*, 2000; Nehme *et al.*, 2007; Buchon *et al.*, 2013). Alteration in the gut microbial homeostasis is known to influence insect behavior (Sharon *et al.*, 2010) and probably also affects insect performance. We hypothesize that increasing dietary carbohydrates could negatively impact *M. sexta* growth in two ways. The ingestion of high levels of carbohydrates might increase the size of microbial communities to levels that first outcompete *M. sexta* for limiting nutrients, such as nitrogen, and, second, alter the microbial community homeostasis so as to increase the prevalence of pathogenic microorganisms. Consistent with the first hypothesis, we found that the negative effects of ingesting an excess of dietary carbohydrates on *M. sexta* growth were reversed by increasing the amount of dietary protein. It remains to be investigated to what extent this process might be driven by changes in the *M. sexta* gut microbial community.

We found that the efficiency of conversion of ingested food tended to increase with increasing dietary protein concentration and, as a consequence, *M. sexta* might have been able to cope better with excess dietary carbohydrates. Protein quality and quantity are subject to considerable variation (Bloem & Duffey, 1990; Felton, 1996), and proteins can interact with plant secondary chemistry to affect digestibility (Zucker, 1983). Trypsin proteinase inhibitors (TPIs) could also modulate the effect of dietary protein content on tissue digestibility. It is worth mentioning that locusts have been shown to perform better on low-nitrogen plants (Cease *et al.*, 2012), indicating species-specific responses to this nutritional parameter. An experimental approach to manipulate protein levels *in planta* is to target RuBisCO, one of the most abundant leaf proteins (Felton, 1996; Taiz & Zeiger, 1998) and one of the main dietary proteins for herbivores (Felton, 1996, 2005). Mitra & Baldwin (2008) reduced the transcript levels of RuBisCO in *N. attenuata* by an *Agrobacterium*-mediated transformation, resulting in a decrease in this protein of up to 1.5-fold. Silencing the expression of this gene, together with reducing PAR and RCA transcripts, and/or sugar supplementation to semi-artificial diets, may allow for a better understanding of the contribution of protein, carbohydrates and their ratios to insect performance in a plant secondary chemistry context. It is important to note that the negative effect of sugars on caterpillar growth was only inverted when protein levels were increased significantly beyond those typically found in leaves. We therefore expect the magnitude, but not the direction, of the jasmonate-dependent sugar depletion effect to change with more modest changes in protein levels.

The results of our study are of potential significance for the evolution of plant defense syndromes. Natural *N. attenuata* populations exhibit high phenotypic plasticity in herbivory-induced jasmonate production (Machado *et al.*, 2013), which is positively correlated with secondary metabolite biosynthesis (Gaquerel *et al.*, 2009) and might therefore be correlated with herbivore resistance (Royo *et al.*, 1999; Halitschke & Baldwin, 2003; Li *et al.*, 2004; Kallenbach *et al.*, 2012). We found that jasmonate deficiency leads to higher sugar concentrations in leaves which may, in turn, reduce *M. sexta* performance. Therefore, this jasmonate-dependent sugar depletion might lead to trade-offs that contribute to natural variation in jasmonate signaling in nature and may favor jasmonate-independent resistance mechanisms.

## Acknowledgements

We would like to thank the Max Planck Society for financial support. We would also like to thank the root–herbivore interactions group, the ITB group members and the glasshouse team for their support. Special thanks go to Mareike Schirmer and Melanie Armbruster for technical support. We thank three anonymous reviewers for their helpful comments on a previous version of the manuscript. M.E. was supported by a Marie Curie Intra European Fellowship (grant no. 273107). C.C.M.A. was supported by the Brazilian National Council for Research (CNPq; grant no. 237929/2012-0). A.P.F was supported by an Alexander von Humboldt Postdoctoral Fellowship.

## References

**Abdala G, Castro G, Miersch O, Pearce D. 2002.** Changes in jasmonate and gibberellin levels during development of potato plants (*Solanum tuberosum*). *Plant Growth Regulation* **36**: 121–126.

**Appel HM, Arnold TM, Schultz JC. 2012.** Effects of jasmonic acid, branching and girdling on carbon and nitrogen transport in poplar. *New Phytologist* **195**: 419–426.

**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.

**Arnold TM, Schultz JC. 2002.** Induced sink strength as a prerequisite for induced tannin biosynthesis in developing leaves of *Populus*. *Oecologia* **130**: 585–593.

**Babic B, Poisson A, Darwish S, Lacasse J, Merkx-Jacques M, Despland E, Bede JC. 2008.** Influence of dietary nutritional composition on caterpillar salivary enzyme activity. *Journal of Insect Physiology* **54**: 286–296.

**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.

**Basset A, Khush RS, Braun A, Gardan L, Boccard F, Hoffmann JA, Lemaitre B. 2000.** The phytopathogenic bacterium *Erwinia carotovora* infects *Drosophila* and activates an immune response. *Proceedings of the National Academy of Sciences, USA* **97**: 3376–3381.

**Behmer ST. 2008.** Insect herbivore nutrient regulation. *Annual Review of Entomology* **54**: 165.

**Bennett RN, Wallsgrove RM. 1994.** Secondary metabolites in plant defence mechanisms. *New Phytologist* **127**: 617–633.

**Bhaskar PB, Wu L, Busse JS, Whitty BR, Hamernik AJ, Jansky SH, Buell CR, Bethke PC, Jiang J. 2010.** Suppression of the vacuolar invertase gene prevents cold-induced sweetening in potato. *Plant Physiology* **154**: 939–948.

**Bloem KA, Duffey SS. 1990.** Effect of protein type and quantity on growth and development of larval *Heliothis zea* and *Spodoptera exigua* and the endoparasitoid *Hyposoter exiguae*. *Entomologia Experimentalis et Applicata* **54**: 141–148.

**Bogatek R, Côme D, Corbineau F, Ranjan R, Lewak S. 2002.** Jasmonic acid affects dormancy and sugar catabolism in germinating apple embryos. *Plant Physiology and Biochemistry* **40**: 167–173.

**Bonaventure G, Schuck S, Baldwin IT. 2011.** Revealing complexity and specificity in the activation of lipase-mediated oxylipin biosynthesis: a specific role of the *Nicotiana attenuata* GLA1 lipase in the activation of jasmonic acid biosynthesis in leaves and roots. *Plant, Cell & Environment* **34**: 1507–1520.

**Bradford MM. 1976.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **72**: 248–254.

**Braun DM, Wang L, Ruan YL. 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.

**Buchon N, Osman D, David F, Yu Fang H, Boquete J-P, Deplancke B, Lemaitre B. 2013.** Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. *Cell Reports* **3**: 1725–1738.

**Cease AJ, Elser JJ, Ford CF, Hao S, Kang L, Harrison JF. 2012.** Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. *Science* **335**: 467–469.

**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.

**Chippindale AK, Chu TJ, Rose MR. 1996.** Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* **50**: 753–766.

**Clissold FJ, Tedder BJ, Conigrave AD, Simpson SJ. 2010.** The gastrointestinal tract as a nutrient-balancing organ. *Proceedings. Biological Sciences/The Royal Society* **277**: 1751–1759.

**Cohen R, Friedman S, Waldbauer G. 1988.** Physiological control of nutrient self-selection in *Heliothis zea* larvae: the role of serotonin. *Journal of Insect Physiology* **34**: 935–940.

**Corbineau F, Rudnicki R, Come D. 1988.** The effects of methyl jasmonate on sunflower (*Helianthus annuus* L.) seed germination and seedling development. *Plant Growth Regulation* **7**: 157–169.

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

**van Dam NM, Horn M, Mares M, Baldwin IT. 2001.** Ontogeny constrains systemic protease inhibitor response in *Nicotiana attenuata*. *Journal of Chemical Ecology* **27**: 547–568.

**Diezel C, Allmann S, Baldwin IT. 2011.** Mechanisms of optimal defense patterns in *Nicotiana attenuata*: flowering attenuates herbivory-elicited ethylene and jasmonate signaling. *Journal of Integrative Plant Biology* **53**: 971–983.

**Engel P, Moran NA. 2013.** The gut microbiota of insects – diversity in structure and function. *FEMS Microbiology Reviews* **37**: 699–735.

**Felton GW. 1996.** Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Archives of Insect Biochemistry and Physiology* **32**: 107–130.

**Felton GW. 2005.** Indigestion is a plant's best defense. *Proceedings of the National Academy of Sciences, USA* **102**: 18771–18772.

**Fernstrom JD. 1987.** Food-induced changes in brain serotonin synthesis: is there a relationship to appetite for specific macronutrients? *Appetite* **8**: 163–182.

**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.

**Fragoso V, Rothe E, Baldwin IT, Kim SG. 2014.** Root jasmonic acid synthesis and perception regulate folivore-induced shoot metabolites and increase *Nicotiana attenuata* resistance. *New Phytologist* **202**: 1335–1345.

**Gaquerel E, Weinhold A, Baldwin IT. 2009.** Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphigidae) and its natural host *Nicotiana attenuata*. VIII. An unbiased GCxGC-ToFMS analysis of the plant's elicited volatile emissions. *Plant Physiology* **149**: 1408–1423.

**Gómez S, Ferrieri RA, Schueller M, Orians CM. 2010.** Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytologist* **188**: 835–844.

**Gómez S, Steinbrenner AD, Osorio S, Schueller M, Ferrieri RA, Fernie AR, Orians CM. 2012.** From shoots to roots: transport and metabolic changes in tomato after simulated feeding by a specialist lepidopteran. *Entomologia Experimentalis et Applicata* **144**: 101–111.

**Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wicher D, Hansson BS. 2011.** Antennal transcriptome of *Manduca sexta*. *Proceedings of the National Academy of Sciences, USA* **108**: 7449–7454.

**Halitschke R, Baldwin IT. 2003.** Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. *Plant Journal* **36**: 794–807.

**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.

**Horibe T, Yamaki S, Yamada K. 2013.** Effects of auxin and methyl jasmonate on cut rose petal growth through activation of acid invertase. *Postharvest Biology and Technology* **86**: 195–200.

**Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V, Kogel K-H, Schäfer P. 2011.** Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. *Plant Physiology* **156**: 726–740.

**Jin Y, Ni D-A, Ruan Y-L. 2009.** Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. *Plant Cell* **21**: 2072–2089.

**Kallenbach M, Bonaventure G, Gilardoni PA, Wissgott A, Baldwin IT. 2012.** *Empoasca* leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations. *Proceedings of the National Academy of Sciences, USA* **109**: E1548–E1557.

**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.

**Kotkar HM, Sarate PJ, Tamhane VA, Gupta VS, Giri AP. 2009.** Responses of midgut amylases of *Helicoverpa armigera* to feeding on various host plants. *Journal of Insect Physiology* **55**: 663–670.

**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.

**Landgraf R, Schaarschmidt S, Hause B. 2012.** Repeated leaf wounding alters the colonization of *Medicago truncatula* roots by beneficial and pathogenic microorganisms. *Plant, Cell & Environment* **35**: 1344–1357.

**Lee KP, Raubenheimer D, Behmer ST, Simpson SJ. 2003.** A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker). *Journal of Insect Physiology* **49**: 1161–1171.

**Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA. 2004.** The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell* **16**: 126–143.

**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.

**Meldau S, Wu J, Baldwin IT. 2009.** Silencing two herbivory-activated MAP kinases, SIPK and WIPK, does not increase *Nicotiana attenuata*'s susceptibility to herbivores in the glasshouse and in nature. *New Phytologist* **181**: 161–173.

**Merkx-Jacques M, Bede JC. 2005.** Influence of diet on the larval beet armyworm, *Spodoptera exigua*, glucose oxidase activity. *Journal of Insect Science (Online)* **5**: 1–9.

**Merkx-Jacques M, Despland E, Bede JC. 2008.** Nutrient utilization by caterpillars of the generalist beet armyworm, *Spodoptera exigua*. *Physiological Entomology* **33**: 51–61.

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

**Mitra S, Baldwin IT. 2014.** RuBPCase activase (RCA) mediates growth–defense trade-offs: silencing RCA redirects jasmonic acid (JA) flux from JA-isoleucine to methyl jasmonate (MeJA) to attenuate induced defense responses in *Nicotiana attenuata*. *New Phytologist* **201**: 1385–1395.

**Nägele T, Henkel S, Hörmiller I, Sauter T, Sawodny O, Ederer M, Heyer AG. 2010.** Mathematical modeling of the central carbohydrate metabolism in Arabidopsis reveals a substantial regulatory influence of vacuolar invertase on whole plant carbon metabolism. *Plant Physiology* **153**: 260–272.

**Nakamura Y, Matsubara A, Miyatake R, Okada M, Ueda M. 2006.** Bioactive substances to control nyctinasty of Albizzia plants and its biochemistry. *Regulation of Plant Growth & Development* **41**(Suppl): 44.

**Nehme NT, Liégeois S, Kele B, Giammarinaro P, Pradel E, Hoffmann JA, Ewbank JJ, Ferrandon D. 2007.** A model of bacterial intestinal infections in *Drosophila melanogaster*. *PLoS Pathogens* **3**: e173.

**Ohyama A, Hirai M. 1999.** Introducing an antisense gene for a cell-wall-bound acid invertase to tomato (*Lycopersicon esculentum*) plants reduces carbohydrate content in leaves and fertility. *Plant Biotechnology* **16**: 147–151.

**Onkokesung N, Gaquerel E, Kotkar H, Kaur H, Baldwin IT, Galis I. 2012.** MYB8 controls inducible phenolamide levels by activating three novel hydroxycinnamoyl-coenzyme A:polyamine transferases in *Nicotiana attenuata*. *Plant Physiology* **158**: 389–407.

**Paschold A, Halitschke R, Baldwin I. 2007.** Co (i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. *Plant Journal* **51**: 79.

**Pohlon E, Baldwin IT. 2001.** Artificial diets 'capture' the dynamics of jasmonate-induced defenses in plants. *Entomologia Experimentalis et Applicata* **100**: 127–130.

**Pozo MJ, Azcón-Aguilar C. 2007.** Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* **10**: 393–398.

**R Development Core Team. 2012.** *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

**Raines CA. 2003.** The Calvin cycle revisited. *Photosynthesis Research* **75**: 1–10.

**Raubenheimer D. 1992.** Tannic acid, protein, and digestible carbohydrate: dietary imbalance and nutritional compensation in locusts. *Ecology* **73**: 1012–1027.

**Raubenheimer D, Lee K, Simpson S. 2005.** Does Bertrand's rule apply to macronutrients? *Proceedings. Biological Sciences/The Royal Society* **272**: 2429–2434.

**Raubenheimer D, Simpson S. 1990.** The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behaviour of larval *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal). I. Short-term studies. *Physiological Entomology* **15**: 219–233.

**Raubenheimer D, Simpson SJ. 1997.** Integrative models of nutrient balancing: application to insects and vertebrates. *Nutrition Research Reviews* **10**: 151–179.

**Rayapuram C, Baldwin IT. 2006.** Using nutritional indices to study LOX3-dependent insect resistance. *Plant, Cell & Environment* **29**: 1585–1594.

**Roeder KA, Behmer ST. 2014.** Lifetime consequences of food protein–carbohydrate content for an insect herbivore. *Functional Ecology* **28**: 1135–1143.

**Rolland F, Baena-Gonzalez E, Sheen J. 2006.** Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**: 675–709.

**Royo J, León J, Vancanneyt G, Albar JP, Rosahl S, Ortego F, Castañera P, Sánchez-Serrano JJ. 1999.** Antisense-mediated depletion of a potato lipoxygenase reduces wound induction of proteinase inhibitors and increases weight gain of insect pests. *Proceedings of the National Academy of Sciences, USA* **96**: 1146–1151.

**Ruiz J, Romero L. 1999.** Nitrogen efficiency and metabolism in grafted melon plants. *Scientia Horticulturae* **81**: 113–123.

**Salem H, Bauer E, Strauss AS, Vogel H, Marz M, Kaltenpoth M. 2014.** Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host. *Proceedings. Biological Sciences/The Royal Society* **281**: 20141838.

**Salem H, Kreutzer E, Sudakaran S, Kaltenpoth M. 2013.** Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). *Environmental Microbiology* **15**: 1956–1968.

**Schwachtje J, Minchin PE, Jahnke S, van Dongen JT, Schittko U, Baldwin IT. 2006.** SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proceedings of the National Academy of Sciences, USA* **103**: 12935–12940.

**Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E. 2010.** Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **107**: 20051–20056.

**Shoji T, Ogawa T, Hashimoto T. 2008.** Jasmonate-induced nicotine formation in tobacco is mediated by tobacco COI1 and JAZ genes. *Plant and Cell Physiology* **49**: 1003–1012.

**Simpson SJ, Raubenheimer D. 2001.** The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. *Ecology* **82**: 422–439.

**Simpson SJ, Raubenheimer D. 2009.** Macronutrient balance and lifespan. *Aging* **1**: 875.

**Simpson SJ, Sibly RM, Lee KP, Behmer ST, Raubenheimer D. 2004.** Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour* **68**: 1299–1311.

**Skrzypek E, Miyamoto K, Saniewski M, Ueda J. 2005.** Jasmonates are essential factors inducing gummosis in tulips: mode of action of jasmonates focusing on sugar metabolism. *Journal of Plant Physiology* **162**: 495–505.

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

**Staswick PE, Su W, Howell SH. 1992.** Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proceedings of the National Academy of Sciences, USA* **89**: 6837–6840.

**Stein E, Molitor A, Kogel K-H, Waller F. 2008.** Systemic resistance in Arabidopsis conferred by the mycorrhizal fungus *Piriformospora indica* requires jasmonic acid signaling and the cytoplasmic function of NPR1. *Plant and Cell Physiology* **49**: 1747–1751.

**Steinbrenner AD, Gómez S, Osorio S, Fernie AR, Orians CM. 2011.** Herbivore-induced changes in tomato (*Solanum lycopersicum*) primary metabolism: a whole plant perspective. *Journal of Chemical Ecology* **37**: 1294–1303.

**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.

**Sturm A, Tang G-Q. 1999.** The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Science* **4**: 401–407.

**Taiz L, Zeiger E. 1998.** *Plant physiology*. Sunderland, MA, USA: Sinauer Inc.

**Tang G-Q, Lüscher M, Sturm A. 1999.** Antisense repression of vacuolar and cell wall invertase in transgenic carrot alters early plant development and sucrose partitioning. *Plant Cell* **11**: 177–189.

**Telang A, Buck NA, Chapman RF, Wheeler DE. 2003.** Sexual differences in postingestive processing of dietary protein and carbohydrate in caterpillars of two species. *Physiological and Biochemical Zoology* **76**: 247–255.

**Thompson S, Redak R. 2000.** Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta*. *Biochimica et Biophysica Acta (BBA)–General Subjects* **1523**: 91–102.

**Tytgat TO, Verhoeven KJ, 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.

**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.

**Waldbauer G, Friedman S. 1991.** Self-selection of optimal diets by insects. *Annual Review of Entomology* **36**: 43–63.

**Waldbauer GP. 1968.** The consumption and utilization of food by insects. *Advances in Insect Physiology* **5**: 229–288.

**Wang L, Allmann S, Wu J, Baldwin IT. 2008.** Comparisons of LIPOXYGENASE3-and JASMONATE-RESISTANT4/6-silenced plants reveal that jasmonic acid and jasmonic acid–amino acid conjugates play different roles in herbivore resistance of *Nicotiana attenuata*. *Plant Physiology* **146**: 904–915.

**Wang W, Liu G, Niu H, Timko MP, Zhang H. 2014.** The F-box protein COI1 functions upstream of MYB305 to regulate primary carbohydrate metabolism in tobacco (*Nicotiana tabacum* L. cv. TN90). *Journal of Experimental Botany* **65**: 2147–2160.

**Warbrick-Smith J, Behmer ST, Lee KP, Raubenheimer D, Simpson SJ. 2006.** Evolving resistance to obesity in an insect. *Proceedings of the National Academy of Sciences, USA* **103**: 14045–14049.

**Wu J, Baldwin IT. 2010.** New insights into plant responses to the attack from insect herbivores. *Annual Review of Genetics* **44**: 1–24.

**Zanotto F, Gouveia S, Simpson S, Calder D. 1997.** Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *Journal of Experimental Biology* **200**: 2437–2448.

**Zhang H, Hedhili S, Montiel G, Zhang Y, Chatel G, Pré M, Gantet P, Memelink J. 2011.** The basic helix-loop-helix transcription factor CrMYC2 controls the jasmonate-responsive expression of the ORCA genes that regulate alkaloid biosynthesis in *Catharanthus roseus*. *Plant Journal* **67**: 61–71.

**Zrenner R, Schüler K, Sonnewald U. 1996.** Soluble acid invertase determines the hexose-to-sucrose ratio in cold-stored potato tubers. *Planta* **198**: 246–252.

**Zucker WV. 1983.** Tannins: does structure determine function? An ecological perspective. *The American Naturalist* **121**: 335–365.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Silencing RuBisCO activase (RCA) reduces sugar concentrations in the leaves at the end of the dark phase.

**Fig. S2** Soluble protein concentrations remain unaltered in response to simulated and actual *Manduca sexta* herbivory in empty vector (EV), inverted repeat allene oxide cyclase (irAOC), inverted repeat RuBisCO activase (irRCA) and irAOC × irRCA plants.

**Fig. S3** Photosynthetically active radiation (PAR) reduction does not alter temperature and humidity in the plant headspace, but slightly reduces red : far-red ratios.

**Fig. S4** No evidence of shade avoidance responses in photosynthetically active radiation (PAR)-reduced plants.

**Notes S1** Detailed statistical tests.

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.