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Research

Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato

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Summary

- Evidence is emerging to support the notion that in response to herbivory, plants undergo changes in their primary metabolism and are able to fine-tune the allocation of new and existing resources and temporarily direct them to storage organs.
- We hypothesized that simulated herbivory increases the export of resources out of the affected tissues and increases allocation to roots. We used short-lived radioisotopes to study *in vivo* the dynamics of newly incorporated $^{11}CO_2$ and $^{13}NH_3$. Methyl jasmonate (MeJA), a known defense elicitor, was applied to the foliage of tomato plants and 4 h later we monitored leaf uptake, export and whole-plant allocation of [^{11}C]photosynthate and [^{13}N]amino acids.
- There was a marginally significant decrease in the fixation of $^{11}CO_2$, and an increase in the export of newly acquired carbon and nitrogen out of MeJA-treated leaves. The proportion of nitrogen allocated to roots increased, whereas the proportion of carbon did not change.
- These results are in agreement with our hypotheses, showing a change in the allocation of resources after treatment with MeJA; this may reduce the chance of resources being lost to herbivores and act as a buffer to biotic stress by increasing the potential for plant regrowth and survival after the attack.

Introduction

Plants are able to perceive and respond to a wide range of biotic and abiotic stimuli (Metlen *et al.*, 2009). In response to these stimuli they undergo physiological, biochemical and physical changes to produce a phenotype that matches their environment (Sultan, 2000). Such phenotypic plasticity can be expressed locally at the site affected by the stimuli. However, plants can also coordinate their responses to changes in their surroundings with other plant modules and respond in a systemic and integrated manner at the whole-plant level (de Kroon *et al.*, 2005).

Upon attack by herbivores, plants produce a number of defensive compounds and structures that hinder the performance and fitness of the attackers (Karban & Baldwin, 1997). This is a preventive characteristic that confers protection against a likely subsequent attack. In tomato, this includes the production of proteinase inhibitors (Farmer & Ryan, 1990; Orians *et al.*, 2000), polyphenol oxidase (Constabel *et al.*, 1995; Mahanil *et al.*, 2008), phenolic compounds (Stamp & Yang, 1996), glycoalkaloids (Duffey & Stout, 1996) and trichomes (Peiffer *et al.*, 2009). However, the plant's arsenal to fight herbivores has associated costs, related to the biosynthesis, maintenance, storage and construction of structures, as well as indirect ecological costs (Bergelson & Purrington, 1996; Heil & Baldwin, 2002; Strauss *et al.*, 2002). Investment in defense requires the use of valuable resources, which, as a result, might not be available for other important plant functions such as growth or reproduction (Herms & Mattson, 1992).

Besides being costly, the induction of secondary metabolites to act as herbivore deterrents may not always be an efficient and/or desirable option for the plant. Specialist herbivores can use their host's defense compounds for their own protection by accumulating them in their body as they consume the tissue (Nishida, 2002). For instance, Monarch butterfly caterpillars (*Danaus plexippus*) can sequester cardenolides from their milkweed hosts (*Asclepias* spp.), thus conferring them, in turn, with protection against their predators (Malcolm & Brower, 1989). Furthermore, specialist

herbivores may also deal with their host's defense mechanisms through adapted physiological systems to detoxify secondary compounds (Wittstock *et al.*, 2004). Such is the case for the voracious *Manduca sexta* caterpillars, which can completely defoliate their natural host, *Nicotiana attenuata*, as a result of their high adaptation to nicotine, a potent neurotoxin against most herbivores (Morris, 1984; Wink & Theile, 2002). Nicotine is an expensive mode of defense (Baldwin, 1998; van Dam & Baldwin, 1998). Thus, in response to damage by *Manduca*, nicotine production is decreased in tobacco plants, and the synthesis of other defense compounds takes place (Kahl *et al.*, 2000). In situations where a herbivore can exploit or overcome the host-defense mechanisms, plants must employ alternative or complementary strategies to withstand the attack.

Plants may use tolerance as a strategy against herbivores, whereby stored resources sustain plant regrowth and reproduction after herbivory without having a direct, negative effect on the herbivores (Mauricio *et al.*, 1997). Both defense and tolerance are effective strategies for reducing the negative impact of herbivores (Stowe *et al.*, 2000). Defoliation by specialist herbivores might be very severe (Harrison & Thomas, 1991) and too fast for the plant to implement an effective defense response. In such cases, tolerance might be the only chance for survival. Tolerance and defense are not mutually exclusive strategies; however, the one that prevails probably depends on a combination of intrinsic and extrinsic factors (Mauricio *et al.*, 1997; Strauss & Agrawal, 1999; Leimu & Koricheva, 2006).

Tolerance is probably the result of a number of traits that are inter-related in a complex manner and its mechanisms are poorly understood. Some mechanisms that are thought to be involved in plant tolerance to herbivores include increased rates of photosynthesis in leaves, activation of dormant meristems and the use of stored resources (Strauss & Agrawal, 1999; Tiffin, 2000). The reallocation of resources stored in roots to shoots is probably the most common mechanism associated with tolerance in herbaceous species (Welter & Steggall, 1993; Zangerl *et al.*, 1997; de Jong & van der Meijden, 2000). It has recently been shown that short-term reallocation of resources away from the site of damage and into storage organs occurs after herbivory (Babst *et al.*, 2005, 2008; Schwachtje *et al.*, 2006). Schwachtje *et al.* (2006) showed a 10% increase in newly fixed carbon (C) transported towards roots in *Nicotiana attenuata* 5 h after simulated herbivory and suggested that this increase in C was later used to sustain seed production and to delay senescence. Investment in reproduction after damage is in agreement with the results of Haukioja & Koricheva (2000) who predict that herbaceous species will allocate stored resources to this function after herbivory instead of the replacement of vegetative tissues, as expected for woody species.

From the perspective of a herbivore, nitrogen (N) is often a limiting element in their diets (Mattson, 1980), making plant N-allocation patterns in response to attack crucial for an effective response in order to reduce its availability to the attacker. From the plant's perspective, N plays a central role in primary and secondary metabolism; therefore, minimizing loss to herbivores, and optimizing partition between plant functions, will maximize plant performance and fitness. While short-term changes in C dynamics have been the focus of recent studies (Babst *et al.*, 2005, 2008; Schwachtje *et al.*, 2006), short-term N dynamics in response to herbivory is an important component in the plant's ability to withstand an attack and needs to be investigated at the whole-plant level.

The aim of our study was to investigate the dynamics of the recent uptake of C and N in tomato (*Solanum lycopersicum* cv First Lady II F1) in response to simulated herbivory. Methyl jasmonate (MeJA) is a well-known defense elicitor that is often used to mimic the effects of wounding by herbivores (Gundlach *et al.*, 1992; McConn *et al.*, 1997; Baldwin & Hamilton, 2000; Van Dam *et al.*, 2001; Pauwels *et al.*, 2009) and was used to simulate herbivore attack in our study. We hypothesized that a simulated herbivore attack would result in a change in resource dynamics, favoring the mobilization of newly acquired resources away from the site of attack and into organs inaccessible to folivores. More specifically, we expected to find an increase in the export of C out of the leaves and increased C allocation to the roots, in MeJA-treated plants. Likewise, we predicted increased export of N from the leaves into the roots. We used the short-lived radioisotopes ^{11}C as $^{11}CO_2$ ($t_{½}$ = 20.4 min) and ^{13}N as $^{13}NH_3$ ($t_{½}$ = 9.97 min) to monitor the uptake, transport and allocation of [^{11}C] photosynthate and [^{13}N]amino acids within the plant. The short-lived nature of these isotopes allows the same plants to be labeled with radioisotopes and radioactivity measured, before and after treatment, in a nondestructive manner.

Materials and Methods

Plant material

Tomato seeds (*Solanum lycopersicum* L. cv First Lady II F1; Hazzard's Greenhouse, Deford, MI, USA) were sown in potting soil (Pro-mix; Premier Horticulture Inc., Quakertown, PA, USA) containing slow-release fertilizer (Osmocote plus 15-9-12; The Scotts Company, Marysville, OH, USA). The mean height of the plants used in the experiments was 28.5 ± 0.5 cm, and they had, on average, five fully developed and three developing leaves. Plants were grown under metal-halide lamps (at a light intensity of 350 μmol m^{-2} s^{-1}) at 24°C with a 16 h : 8 h light/dark photoperiod.

Experimental set-up

Each plant was placed individually in a lighted hood equipped for radiotracer administration. Three radiation detectors were used to measure activity in different areas of the plant. The radioactivity measured by the detectors indicates the amount of radioisotope (either ^{11}C or ^{13}N) leaving the focal leaf where it was administered and moving into other plant areas as [^{11}C]photosynthate or [^{13}N]amino acids, respectively (see later; Fig. 1).

The plant stem was aligned with a detector situated directly above the apex and a detector pointing at the root area. A third detector was placed on the leaf cell where the radiotracer was administered. Differences in plant height, morphology and positioning within the chamber were corrected for before tracer administration by using an encapsulated ^{68}Ge radioactive source that decays to a radioactive daughter, ^{68}Ga. Both isotopes are in secular equilibrium and emit 511 KeV gamma rays through positron annihilation. The source was *c.* 0.1 μCi. The radioactive source was positioned on the plant apical meristem and on the base of the stem where the roots start to develop. The background counts were subtracted from the counts measured from the radioactive source in the apex and the root area. After the background correction, the root counts were divided by the apex counts, obtaining a correction factor used to adjust the data collected during radiotracer administration. The correction factor was multiplied by the data collected from the apex detector during the experiment, allowing comparison of readings acquired by both the root and apex detectors.

After calibration, an airtight cell (5 cm × 10 cm) was placed over two leaflets of the third-youngest fully developed leaf, allowing the administration of radiotracers only to the area enclosed in the cell (Ferrieri *et al.*, 2005). A light source was placed directly on top of the leaf cell (light intensity: 920 μmol m^{-2} s^{-1}) with an attached water-based cooling system to avoid excessive heat. The plant was set up in the hood for 30–60 min before administration of the radiotracer.

Each plant received radiotracers – $^{11}CO_2$ or $^{13}NH_3$ – before (day 1) and after (day 2) treatment with MeJA or control spray, as described in the section 'Radiotracer administration'. Plants were only used once and there were five or six replicate plants per radiotracer type per treatment.

Radiotracer administration

The use of short-lived radiotracers allows nondestructive measurements to be made on the same plant before and after treatment. Each plant was treated with either $^{13}NH_3$ ($t_{½}$ = 9.97 m) or $^{11}CO_2$ ($t_{½}$ = 20.4 m). $^{11}CO_2$ was produced via $^{14}N(p,\alpha)^{11}C$ nuclear transformation (Ferrieri & Wolf, 1983) from a 50-ml volume high-purity N gas target using 17 MeV protons from the TR-19 (Ebco Industries Ltd, Richmond, BC, Canada) cyclotron at Brookhaven National Laboratory, and captured on a molecular sieve (4 Å) (full details in Ferrieri *et al.*, 2005). $^{13}NH_3$ was produced via $^{16}O(p,n)^{13}N$ nuclear transformation from distilled water using a 2.5-ml volume high-pressure liquid target and 17 MeV protons on the same cyclotron used for $^{14}N(p,\alpha)^{11}C$ nuclear transformation. The ^{13}N was

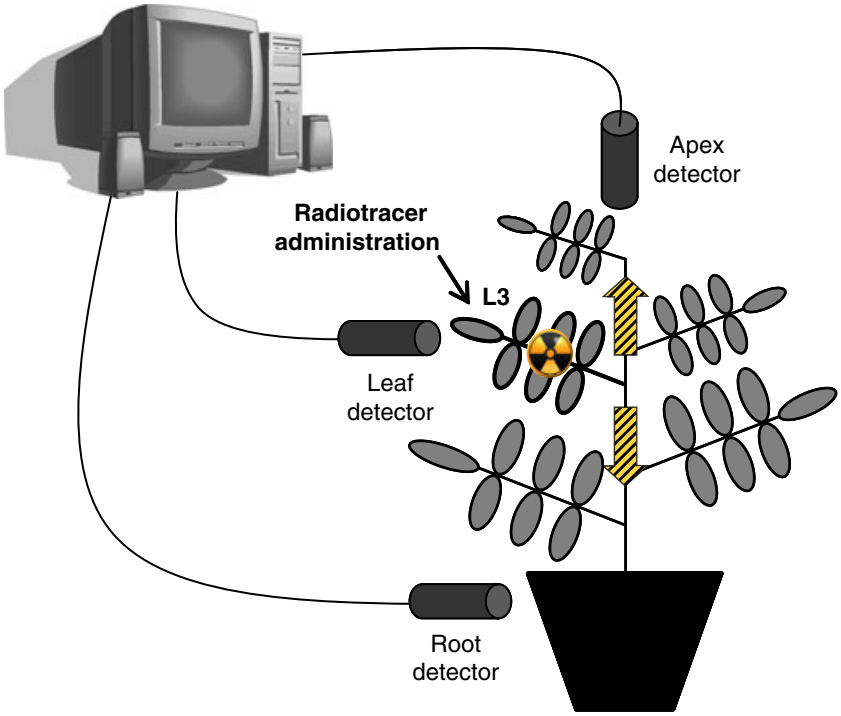

Fig. 1 Schematic experimental set-up of the radiotracer labeling process. Two tomato leaflets from leaf 3 (L3) were enclosed in an airtight cell where the radiotracer was administered. The arrows represent the movement of the tracer within the plant once introduced into the plant. The scintillation detectors (cylinders) were connected to a computer, providing measurements in real time.

recovered as $^{13}NO_3^-$ and chemically reduced to $^{13}NH_3$ gas using DeVarda's Alloy (Aldrich Chemical, St Louis, MI, USA) (Ferrieri & Wolf, 1983).

$^{11}CO_2$ and $^{13}NH_3$ are introduced into the plant metabolic machinery in a gaseous form and converted into other mobile compounds, which are then translocated from the load leaf to other tissues through the vascular system. $^{11}CO_2$ is introduced into the plant via the Calvin cycle, being mainly [^{11}C]sugars, and the mobile end product was tracked in our experiments. $^{13}NH_3$ is assimilated by glutamine-synthetase, resulting in the synthesis of mobile [^{13}N]amino acids (R. Ferrieri, unpublished).

The plants were locally radiolabeled on two consecutive days. On day 1 ('Baseline'), baseline measurements were taken and no treatment was applied. On day 2 ('Post-treatment'), the plants were radiolabeled at the same time as on day 1, but treated for 4 h before radiolabeling. The radiotracer was administered on the third fully developed leaf (L3) counting from the apex (Fig. 1).

The radioactivity was monitored for 2 h after radiotracer administration, producing real-time readings in different tissues. A PIN diode radiation detector (Bioscan Inc., Washington, DC, USA) fixed to the bottom of the airtight cell on L3, and two sodium-iodide scintillation detectors (Ortec, Oak Ridge, TN, USA) positioned on the apex and root area, respectively, were used to record the following: uptake of the ^{11}C radiotracer by L3 (i.e. $^{11}CO_2$ fixation), which was calculated as the percentage of the amount of $^{11}CO_2$ that was fixed at the end of the radiotracer pulse; export of the radiotracer from L3, which was calculated as the percentage of activity that left the labeled leaf after 2 h; and relative allocation of the radiotracer to the apex and roots, which was calculated as the percentage of the total activity allocated to either the apex or the root tissues. All calculations were performed using activity decay-corrected values to take into account the decay of the radioisotope over the time course of the experiment.

MeJA treatment

Methyl jasmonate is a known plant defense elicitor which up-regulates the production of defensive compounds against herbivores. In tomato, MeJA leads to the production of defense compounds, resulting in reduced tissue digestibility and palatability (Farmer & Ryan, 1990; Constabel *et al.*, 1995; Li *et al.*, 2002). Using MeJA, rather than damage or defoliation, confers the advantage of avoiding confounding effects that tissue damage or removal can have on reallocation patterns as a result of physical resource imbalance. In this study we sprayed plants homogenously aboveground with deionized water containing 0.5 mM MeJA. The control plants were either sprayed aboveground with deionized water or left untreated. The plants were treated 4 h before administration of the radiotracer on day 2.

Data analysis

To control for differences between plants, paired *t*-tests using matched baseline and post-treatment values in each plant were used to analyze treatment effects on radiotracer uptake, export and allocation between apex and roots. All analyses were performed using SAS (GLM PROC TTEST) statistical package version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Resource uptake

[^{11}C]Photosynthate Treatment with MeJA had a marginally significant effect on the fixation of $^{11}CO_2$ ($n = 6$, $P = 0.057$). In the post-treatment measurements (4 h after the application of MeJA), the target leaf used for radiotracer administration showed a decrease in the amount of $^{11}CO_2$ fixed, from an average of 65.1% of the total $^{11}CO_2$ administered before MeJA treatment to 51.6% after MeJA treatment. No significant differences in the fixation of $^{11}CO_2$ were observed in either untreated ($n = 5$) or water-sprayed ($n = 5$) plants (Fig. 2) between baseline and post-treatment values.

Leaf export

[^{11}C]Photosynthate Increased export of [^{11}C]photosynthate was measured in the load leaf in MeJA-treated plants (Fig. 3a). Of the total $^{11}CO_2$ fixed by the leaf, 36.2% was exported as [^{11}C]photosynthate 4 h after treatment with MeJA compared with only 27.1% during the baseline measurements ($n = 6$, $P = 0.003$). No changes in leaf export were detected for either untreated or water-sprayed plants.

[^{13}N]Amino acids Treatment with MeJA had a significant effect on the export of [^{13}N]amino acids out of the load leaf ($n = 5$, $P = 0.016$; Fig. 3b). Of the total recently assimilated N on the leaf, 12.4% was exported during baseline measurements. Leaf export almost doubled after treatment with MeJA, reaching an average value of 22.7%. [^{13}N]Amino acid export out of the load leaf did not change significantly between baseline and post-treatment values in untreated and water-sprayed plants.

Root allocation

[^{11}C]Photosynthate Allocation of [^{11}C]photosynthate between the roots and the apex did not change significantly in either of the experimental groups. There was a trend showing an increase of [^{11}C]photosynthate allocation towards the root compared with the apex area in response to the application of MeJA to the foliage; however, the effect was not significant (Fig. 4a).

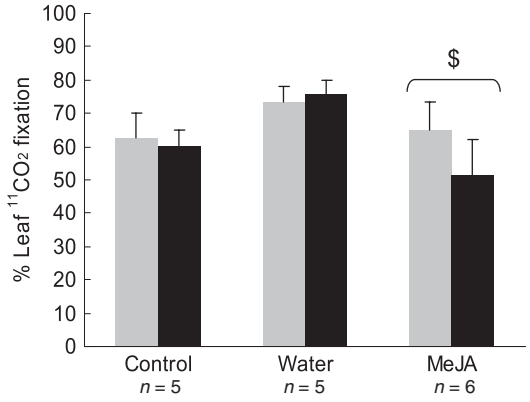

Fig. 2 Percentage of the total $^{11}CO_2$ administered to leaf 3 (L3) that was fixed by the tomato plant during baseline measurements (gray bars) and after treatment with methyl jasmonate (black bars). Bars represent mean + SE. The number of replicates is indicated under each treatment. Paired *t*-tests were used to analyze the data, comparing baseline and post-treatment values in each individual plant. $, $0.05 < P < 0.1$.

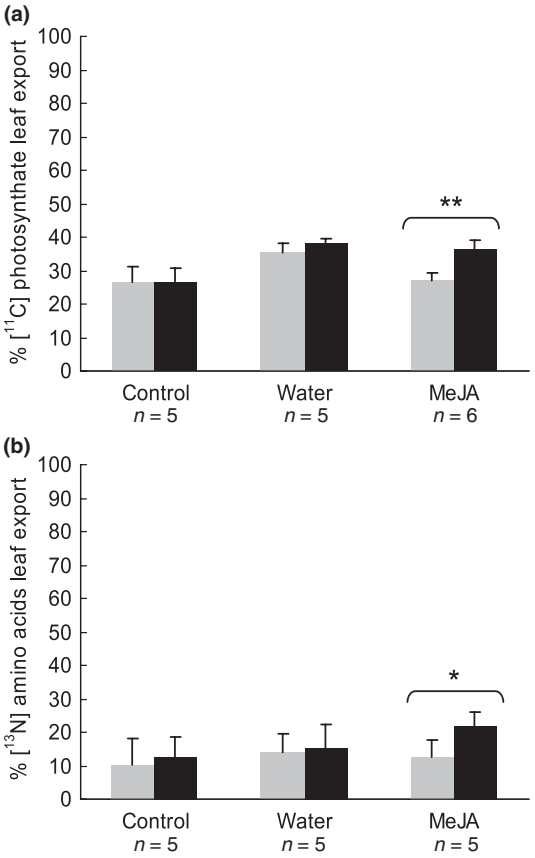

Fig. 3 Percentage of total newly acquired carbon (a) and nitrogen (b) exported by tomato leaf 3 (L3) at baseline (gray bars) and after treatment with methyl jasmonate (black bars). Bars represent mean + SE. The number of replicates is indicated under each treatment. Paired *t*-tests were used to analyze the data comparing baseline and post-treatment values in each individual plant. Only statistically significant values are indicated. *, $P < 0.05$; **, $P < 0.01$.

[^{13}N]Amino acids The relative amount of [^{13}N]amino acids allocated to the roots significantly increased in response to treatment with MeJA ($n = 6$, $P = 0.019$; Fig. 4b). The average percentage of the total activity measured in both roots and apex that was allocated to roots was 41.5% during baseline measurements and increased to 61.2% after treatment with MeJA. Root allocation did not change in untreated or water-sprayed plants.

Discussion

Our results showed that MeJA induces rapid effects on tomato resource dynamics. Within 4 h of MeJA application onto the foliage of tomato plants, changes in resource uptake, export and allocation patterns were observed. Despite the advantages of short-lived isotopes, very few studies have used ^{11}C to examine photosynthate movement in plants in response to (simulated) herbivory (Babst *et al.*, 2005, 2008; Schwachtje *et al.*, 2006; Henkes *et al.*, 2008). To the best of our knowledge, this is the first time that ^{13}N,

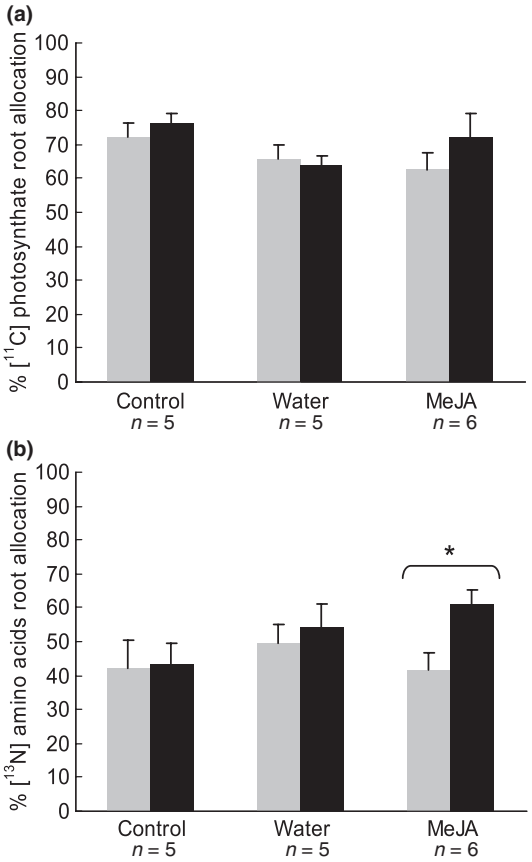

Fig. 4 Percentage of total newly acquired carbon (a) and nitrogen (b) allocated to the tomato roots at baseline (gray bars) and after treatment with methyl jasmonate (black bars). Bars represent mean + SE. The number of replicates is indicated under each treatment. Paired *t*-tests were used to analyze the data comparing baseline and post-treatment values in each individual plant. Only statistically significant values are indicated. *, $P < 0.05$.

introduced as $^{13}NH_3$, has been used to study leaf export and allocation of radiolabeled amino acids in response to MeJA. As leaf-chewing herbivores induce jasmonates (de Vos *et al.*, 2005), our results, using MeJA, suggest that herbivores will also induce both the export and allocation of resources from damaged leaves to roots.

MeJA reduces $^{11}CO_2$ fixation in treated leaves

MeJA-treated plants were found to have a marginally significant (24%) reduction in their photosynthetic capacity, measured as the amount of $^{11}CO_2$ fixed by a focal leaf, compared with untreated, or water-sprayed, plants. Reduced fixation of $^{11}CO_2$ by source leaves can have a negative effect on the development and induction of the synthesis of defense compounds in sink leaves (Arnold & Schultz, 2002; Arnold *et al.*, 2004), which rely on C import. Furthermore, young leaves are frequently preferred by herbivores as a result of their higher nutrient content and reduced toughness (Mckey, 1974; Fenner *et al.*, 1999; Coley *et al.*, 2006) making them even more vulnerable to subsequent attack. A decreased photosynthetic capacity may have a negative impact on growth (Schmidt *et al.*, 2009), affecting plant fitness and performance, especially in the presence of competing neighbors.

A reduction in photosynthesis in response to MeJA is in accordance with a number of other physiological and molecular studies (Creelman & Mullet, 1997; Beltrano *et al.*, 1998; Hristova & Popova, 2002; Rossato *et al.*, 2002; Izaguirre *et al.*, 2003 but see Velitchkova & Fedina, 1998; Moore *et al.*, 2003). Hermsmeier *et al.* (2001) showed that transcripts involved in photosynthesis were strongly down-regulated in *N. attenuata*, while those responding to stress, wounding and pathogens were strongly up-regulated. Importantly, most of the transcripts responded similarly to MeJA and to *M. sexta* caterpillars. A similar reduction in photosynthesis was reported after the application of MeJA on Scots pine (Heijari *et al.*, 2005). Decreased rates of photosynthesis have also been shown to be coupled with a decrease in the chlorophyll content (Hristova & Popova, 2002) and a remobilization of N compounds from senescing leaves to roots (Rossato *et al.*, 2002), suggesting an induction of leaf senescence-like processes in response to MeJA, as shown in Beltrano *et al.* (1998).

Export of newly acquired ^{11}C and ^{13}N increases in MeJA-treated leaves

Our results showed a rapid increase in leaf export of [^{11}C]photosynthate and [^{13}N]amino acids in response to MeJA. A similar decrease in sugar and amino acid contents was observed in *Brassica rapa* leaves shortly after treatment of the foliage with MeJA (Liang *et al.*, 2006). The export of resources, and in particular of N, out of the leaves may act as a preventive strategy to safeguard valuable resources by storing them in distant tissues away from folivores. In western wheatgrass, leaf herbivory by grasshoppers resulted in decreases in foliar N and in the concentration of non-structural C of 12% and 11%, respectively, and in a 43% increase in phenolics, which was negatively correlated with grasshopper performance (Redak & Capinera, 1994).

Nitrogen is the limiting resource for many herbivores (Mattson, 1980). Therefore, a decrease in foliar N can result in herbivores having to compensate for a decrease in leaf quality by consuming larger quantities of plant tissue in order to maintain an appropriate intake of N to supply metabolic demands (Raubenheimer, 1992; Lavoie & Oberhauser, 2004). This will result in longer feeding periods, or, if the attacking herbivores are mobile enough, they may selectively move to neighboring plants. Both longer feeding and foraging periods can result in longer development times, which may enhance predation and parasitization risk by natural enemies (Kessler & Baldwin, 2004; Kaplan *et al.*, 2007).

In our study we observed an increase in the export of both newly acquired C and N out of the MeJA-treated leaves; however, the percentage of N exported was considerably larger, being almost double that of pretreatment values. The decreased CO_2-fixation trend observed after treatment with MeJA may have constrained the potential amount of photosynthate available for export out of the treated leaves. Another possibility is that after MeJA treatment there was a rapid metabolic reconfiguration in the treated leaves and that some of the newly acquired C was partitioned to other functions, such as local defense, or to serve as C skeletons for compounds such as amino acids. These differences in ^{11}C and ^{13}N exported out the leaf are likely to lead to an increase in C : N ratios. As there was a much higher export of N, an increase in the C content relative to N is expected, which has been shown to increase the contents of phenolics and α-tomatine in tomato foliage (Hoffland *et al.*, 1999), both of which are known to have antiherbivore properties. Nitrogen availability can affect both direct and indirect plant-defense strategies (Coviella *et al.*, 2002; Orians *et al.*, 2002; Lou & Baldwin, 2004). In cotton, a low content of N increases the amount of herbivore-induced volatile compounds (Schmelz *et al.*, 2003; Olson *et al.*, 2009 but see Gouinguene & Turlings, 2002), which potentially improves indirect plant-defense mechanisms by increasing the likelihood of attracting natural enemies while maintaining low leaf quality and forcing the herbivores to feed for a longer period of time.

MeJA induces rapid allocation of [^{13}N]amino acid towards the roots

Our results showed a significant increase in root allocation of newly synthesized [^{13}N]amino acid and (a nonsignificant

trend) of [^{11}C]photosynthate. This is in agreement with an increasing number of studies in the literature showing that plants can induce the remobilization of resources away from the site of damage shortly after attack (Babst *et al.*, 2005, 2008; Schwachtje *et al.*, 2006; Newingham *et al.*, 2007; Kaplan *et al.*, 2008a; but see Frost & Hunter, 2008). After damage, plants often rely on stored reserves to compensate for the tissue loss (reviewed in Trumble *et al.*, 1993). It has been suggested that remobilizing valuable and limited resources from the site of damage and sequestering them in inaccessible organs is a tolerance strategy against current herbivores (Babst *et al.*, 2005, 2008; Schwachtje *et al.*, 2006; Schwachtje & Baldwin, 2008). Once the herbivore threat is no longer present, the stored reserves can be used to sustain tissue regrowth and reproduction (Iwasa & Kubo, 1997; de Jong & van der Meijden, 2000), which may affect subsequent fitness. Concrete evidence linking herbivory-induced resource sequestration belowground and plant tolerance remains elusive, and more studies are necessary to elucidate the physiological role of induced resource sequestration (Schwachtje & Baldwin, 2008). Induced resource sequestration could also provide a substrate for the biosynthesis of defense compounds produced elsewhere in the plant. Such is the case in tobacco plants, where leaf damage results in the synthesis of nicotine (a N-rich defense molecule) in the roots, which is subsequently transported to the shoot (Tso & Jeffrey, 1957). However, defoliation of tobacco plants by *M. sexta* did not alter root chemistry (alkaloids and phenolics) in a recent study carried out by Kaplan *et al.* (2008b). In a different study, simulated herbivory using oral secretions from *M. sexta* resulted in the transient inhibition of root growth (Hummel *et al.*, 2009), despite the increase in C allocation towards the roots reported by Schwachtje *et al.* (2006). Taken together, these results suggest that an increase in the export of resources towards the roots may have other physiological functions. In alfalfa, treatment with MeJA resulted in increased N partitioning and the accumulation of vegetative storage proteins in tap roots (Meuriot *et al.*, 2004). This was in agreement with the findings of Frost & Hunter (2008), who showed a decrease of 39% in N allocation towards fine roots and an increase in storage in the tap root and stem in response to folivory in red oak seedlings. Thus, temporary storage might be a possible function for the observed increase in N content towards the roots.

Ecological consequences of induced resource remobilization

Despite the benefits that resource sequestration can confer to plants in response to herbivory, exporting resources to distant organs can have far-reaching consequences that may not always have a positive effect on plant performance. For example, a compensatory feeding behavior prompted by reduced leaf quality will result in larger tissue areas being lost, therefore diminishing the plant's photosynthetic potential. The negative impact of defoliation is not only associated with the actual loss of tissue, but also with indirect physiological effects occurring in undamaged leaves (Zangerl *et al.*, 2002). Furthermore, plants are commonly attacked by a myriad of herbivores simultaneously aboveground and belowground (Dicke *et al.*, 2009). The plant rhizosphere is also colonized by mycorrhizas and microorganisms that utilize plant nutrient leakage (Bardgett *et al.*, 1998). Root exudation and microorganism biomass has been shown to increase after clipping and herbivory (reviewed in Bardgett *et al.*, 1998; Henry *et al.*, 2008). It has also been shown that the application of jasmonic acid as a defense elicitor on tomato leaves results in an increased colonization of roots by mycorrhizas, which has been linked to the up-regulation of genes involved in C metabolism in the roots (Tejeda-Sartorius *et al.*, 2008). Both microbes and mycorrhizas can increase nutrient availability, which may have a positive effect on recovery after attack by herbivores. However, increased root exudation and export of resources to the roots may also make the plant more susceptible to belowground pathogens or herbivores, some of which, such as *Diabrotica* spp. (Godfrey *et al.*, 1993), can have devastating consequences for the plant. Kaplan *et al.* (2008a) demonstrated that aboveground defoliation of tobacco plants by leaf-chewing herbivores caused an increase in C allocation to the roots, which resulted in a positive effect on a gall-forming root nematode. Nutritional changes, along with changes in compounds involved in plant defence, may link aboveground and belowground herbivore interactions (Kaplan *et al.*, 2008a; Johnson *et al.*, 2009). Ultimately, the advantage or disadvantage of reallocating resources to roots will depend on whether aboveground or belowground attackers exert the strongest pressure.

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