Temporal Changes in Allocation and Partitioning of New Carbon as $^{11}\text{C}$ Elicited by Simulated Herbivory Suggest that Roots Shape Aboveground Responses in Arabidopsis$^{1[W][OA]}$

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Using the short-lived isotope $^{11}\text{C}$ (t$_{1/2}$ = 20.4 min) as $^{11}\text{CO}_2$, we captured temporal changes in whole-plant carbon movement and partitioning of recently fixed carbon into primary and secondary metabolites in a time course (2, 6, and 24 h) following simulated herbivory with the well-known defense elicitor methyl jasmonate (MeJA) to young leaves of Arabidopsis (Arabidopsis thaliana). Both $^{11}\text{CO}_2$ fixation and $^{11}\text{C}$-photosynthate export from the labeled source leaf increased rapidly (2 h) following MeJA treatment relative to controls, with preferential allocation of radiolabeled resources belowground. At the same time, $^{11}\text{C}$-photosynthate remaining in the aboveground sink tissues showed preferential allocation to MeJA-treated, young leaves, where it was incorporated into $^{11}\text{C}$-cinnamic acid. By 24 h, resource allocation toward roots returned to control levels, while allocation to the young leaves increased. This corresponded to an increase in invertase activity and the accumulation of phenolic compounds, particularly anthocyanins, in young leaves. Induction of phenolics was suppressed in sucrose transporter mutant plants ($suc2$), indicating that this phenomenon may be controlled, in part, by phloem loading at source leaves. However, when plant roots were chilled to 5°C to disrupt carbon flow between above- and belowground tissues, source leaves failed to allocate resources belowground or toward damaged leaves following wounding and MeJA treatment to young leaves, suggesting that roots may play an integral role in controlling how plants respond defensively aboveground.

Plant responses induced by herbivorous insects depend on the coordination of molecular, biochemical, and physiological events (Mithöfer and Boland, 2012). Plant tissues are not attacked uniformly nor are they uniformly responsive to insect herbivores, in part because they are modular organisms made up of source and sink tissues. While sink tissues (e.g. young leaves, flowers, and roots) are usually the major determinants of carbon partitioning in plants, defense induction can also reconfigure primary metabolism to provide additional substrates and energy required to produce secondary metabolites (Schwachtje and Baldwin, 2008). This results in complex patterns of defense that may vary spatially and temporally (Karban and Baldwin, 1997).

The idea that there are significant tradeoffs among growth, reproduction, and defense function in plants dates back to Darwin and has shaped much of modern plant and chemical ecology. Developing fruits, growing tissues, and storage organs are all significant sinks that compete for a common pool of carbon resources in plants. In past studies, for example, the number and size of competing sinks have been shown to strongly influence the success of galling aphids on cottonwood trees (i.e. their ability to enhance the sink strength at the feeding site and out compete other plant sinks for resources; Larson and Whitham, 1997; Wool, 2004). While investigators have acknowledged different responses in defense chemistry and downstream effects on herbivores as plants enter their reproductive stage (Bazzaz et al., 1987; Zangerl and Bazzaz, 1992; Baldwin, 1998; Barton and Koricheva, 2010), there are no clear generalizations regarding the relationship between other naturally occurring competing sinks (young leaves and roots) and plant defense responses. Moreover, recent attempts to develop a conceptual model of changes made to plant resource allocation...
following herbivore attack that takes into account vascular architecture, tissue development, variation over time, and local environmental variation have been impeded by differences in temporal and spatial variation in responses and by the dependency of induction of local defense responses on other plant modules (Hanhimäki and Senn, 1992; Orians and Jones, 2001).

Recent studies utilized the short-lived positron-emitting radioisotope, $^{11}$C ($t_{1/2} = 20.4$ min) as $^{11}$CO$_2$ to study changes made to carbon allocation during plant defense induction (Babst et al., 2005, 2008; Thorpe et al., 2007; Gómez et al., 2010) and to measure changes in their metabolic partitioning into key secondary metabolites (Ferrieri et al., 2005; Hanik et al., 2010). Previous studies using this short-lived isotope have narrowed their focus to short-term dynamics in resource allocation occurring immediately (within hours) following herbivory. These experiments suggest that plants respond to leaf damage by rapidly transporting recently fixed carbon from both damaged and undamaged tissues to roots, a response thought to be employed by plants to tolerate herbivory by bunkering resources away from sites of damage and into storage belowground for later regrowth (Babst et al., 2005, 2008; Schwachtje et al., 2006; Newingham et al., 2007; Frost and Hunter, 2008; Kaplan et al., 2008; Gómez et al., 2010, 2012; Orians et al., 2011).

In contrast, previous studies in *Populus* spp. show that the ability of young, developing leaves to respond defensively relies on their capacity to induce their sink strength for carbon-based resources by increasing the activity of Suc metabolizing enzymes (invertases) and importing carbon toward damaged sites from orthostichous (directly connected) source leaves (Arnold and Schultz, 2002; Arnold et al., 2004). This response was observed at 24 and 48 h following insect damage using stable isotope labeling ($^{13}$C) techniques. Our previous work in the model plant Arabidopsis (*Arabidopsis thaliana*) also demonstrates the movement of a radioactive Glc surrogate, $[^{18}$F]fluoro-2-deoxy-D-Glc, toward wounded leaves where it was incorporated into phenoic glycosides 4 h after simulated herbivory (Ferrieri et al., 2012).

We hypothesized that the movement of $^{11}$C-photosynthate away from damaged sites (to roots) is an immediate response to defense elicitation and is followed by movement toward damaged sites at later time points. If transported carbohydrates serve as building blocks for the production of secondary metabolites in developing sink leaves, we predicted that transport from source to sink would be required as a prerequisite to induction in these young leaves. To determine whether roots are a dominant sink for resources in Arabidopsis and compete with secondary metabolites for recently fixed carbon, we performed root chilling treatments to disrupt carbon flow between above- and belowground tissues. We predicted that this treatment would suppress the flow of carbohydrates to and from roots, thus removing them as a competing sink and increasing carbon precursors available for primary (sugar) and secondary (phenolic acid) metabolisms in young, developing sink leaves. The short-lived nature of $^{11}$C provides a unique opportunity to determine how newly acquired carbon resources are distributed throughout the plant and partitioned into key metabolite pools during times of stress.

**RESULTS**

**Temporal Changes in Fixation and Export of $^{11}$CO$_2$**

To capture temporal changes in Arabidopsis carbon dynamics in response to simulated herbivory, an individual source leave (load leaf [LL]) was pulse labeled with $^{11}$CO$_2$ and $^{11}$C-uptake and export by this leaf were measured at 2, 6, and 24 h following the combination of wounding and MeJA application (referred to as wound/MeJA treatment throughout remaining text) to two young orthostichous leaves (YO).

Diurnal fluctuations in $^{11}$CO$_2$ fixation and $^{11}$C-photosynthate export from the LL were not observed during our time course; there were no significant differences between control plants labeled at 2 h (AM labeling) and 6 h (PM labeling; fixation, $P = 0.2126$; export, $P = 0.3009$). However, 2 h following wound/MeJA application to YO leaves, both fixation and export were significantly elevated (Fig. 1A and B; $P = 0.015$ and $P = 0.000072$, respectively). $^{11}$C-photosynthate export was elevated at 6 h post treatment as well, despite no change in fixation (Fig. 1B; $P = 0.014$). By 24 h, both fixation and export had returned to baseline levels.

**Whole-Plant Allocation of $^{11}$C-Photosynthate in Response to Wound/MeJA**

The increases in $^{11}$CO$_2$ fixation and export observed 2 h following wound/MeJA treatment co-occurred with preferential allocation of radiolabeled resources belowground relative to control plants (see Supplemental Fig. S1; $P = 0.0235$). Of the $^{11}$C-photosynthate remaining
in aboveground sink tissues, we found preferential allocation to the treated YO leaves at 2 h post treatment (Fig. 1, C and D; \( P = 0.0257 \)). This response returned to control levels by 6 h (Fig. 1D; \( P = 0.6573 \)). By 24 h post treatment, resource allocation to roots was comparable to controls (Supplemental Fig. S1), but allocation to the YO leaves increased (Fig. 1D; \( P = 0.0122 \)).

**Partitioning of \(^{13}\text{C}\) into Soluble Sugars**

We observed marginally significant changes in the partitioning of recently fixed \(^{13}\text{CO}_2\) into soluble sugars, including \(^{13}\text{C}\)-Glc and \(^{13}\text{C}\)-Fru across the time course (Supplemental Figs. S2 and S3). For example, in the LL, \(^{13}\text{C}\)-Glc and \(^{13}\text{C}\)-Fru decreased from 2 to 6 h in both treatments (Supplemental Fig. S2, C and D; \(^{13}\text{C}\)-Glc, \( P = 0.051 \); \(^{13}\text{C}\)-Fru, \( P = 0.023 \)) but by 24 h had returned to the 2 h levels. There was no significant effect of wound/MeJA treatment on \(^{13}\text{C}\)-sugars in the LL. In YO leaves, \(^{13}\text{C}\)-Glc and \(^{13}\text{C}\)-Fru also decreased marginally 24 h following wound/MeJA treatment (Supplemental Fig. S3, C and D; \(^{13}\text{C}\)-Glc, \( P = 0.085 \); \(^{13}\text{C}\)-Fru, \( P = 0.078 \)). Wound/MeJA elicited no change to total \(^{13}\text{C}\)-sugars in the LL or YO leaves.

**Partitioning of \(^{13}\text{C}\) into Organic Acids**

In general, YO leaves allocated more \(^{13}\text{C}\)-photosynthate to the production of \(^{13}\text{C}\)-organic acids, including \(^{13}\text{C}\)-phenolic acids and \(^{13}\text{C}\)-cinnamic acid, compared with the older LL (Supplemental Fig. S4). Wound/MeJA treatment to two YO leaves induced total \(^{13}\text{C}\)-phenolic acids (Supplemental Fig. S4A; \( P = 0.0495 \)) and \(^{13}\text{C}\)-cinnamic acid (Supplemental Fig. S5A; \( P = 0.0413 \)) in the LL 2 h following treatment. No changes were detected in YO leaves following wound/MeJA treatments for any time point (Supplemental Figs. S4B and S5B).
Total 12C-Phenolics

The same time course experiment was repeated at a larger scale, in the absence of 11CO2 labeling, to assess changes in the accumulation 12C-phenolics as well as cell wall (CWI) and soluble (SOL) invertase activity. The accumulation of 12C-phenolics was elevated in young leaves as well as in their respective source leaf 24 h following wound/MeJA treatment to young leaves (Supplemental Fig. S6, A and B). When concentrations of various phenolic classes were assayed separately, we specifically found anthocyanins to be induced in all tissue types 24 h following wound/MeJA treatment (Supplemental Fig. S6, D–F). Flavonoid concentrations in leaves were not affected by wound/MeJA treatment (Supplemental Fig. S6, G and H), but were significantly suppressed in roots at 2 and 6 h following treatment aboveground (Supplemental Fig. S6I). By 24 h posttreatment, this pattern was reversed; flavonoids were significantly elevated in roots relative to respective control plants.

Source Constraint on Phenolic Induction

To test whether the flow of phloem-borne signals or resources traveling from sources to sink leaves facilitates changes made to phenolic chemistry in young, sink leaves, responses were measured in plants where export of carbohydrates from source leaves was disrupted. This was accomplished through the use of a Suc transporter insertion mutant (suc2-1, At1G22710), which is compromised in its ability to load carbohydrates in the phloem of source leaves (Gottwald et al., 2000).

Compared with wild-type plants, suc2-1 mutants contained similar levels of total phenolic compounds in young leaves (Fig. 2). In wild-type plants, wound/MeJA elicitation to two young leaves induced the accumulation of total phenolics locally (Fig. 2A; \( P = 0.013 \)) as well as systemically (Fig. 2B; \( P = 0.002 \)). When individual classes of phenolics were analyzed separately, we found that anthocyanins were significantly elevated following treatment (Supplemental Fig. S7). The application of MeJA to suc2-1 plants induced total phenolic compounds locally (Fig. 2A; \( P = 0.042 \)), but not to the same extent as wild-type plants (8% increase in suc2-1 plants compared with 17% increase in the wild type). In contrast to wild-type plants, the concentration of total phenolics did not increase in young, systemic tissues of suc2-1 plants (Fig. 2B; \( P = 0.283 \)). Within these leaves, anthocyanin levels were also different than controls following wound/MeJA treatment of suc2-1 plants (Supplemental Fig. S7B; \( P = 0.967 \)).

Invertase Activity

Additional experiments were conducted to quantify changes made to the activity of CWI and SOL invertases in leaves and roots to provide insight into the mechanism underlying changes in carbon dynamics observed in our labeling studies. In Arabidopsis, invertases are not only the main route of Suc metabolism but are essential for normal plant growth and development (Barratt et al., 2009). Invertases also play a crucial role in regulating the supply of photosynthate to naturally occurring sink tissues (Tang et al., 1999; Wesche et al., 2003; Heyer et al., 2004; Roitsch and González, 2004) and are known to be up-regulated during gall formation (Rehll and Schultz, 2003; Siemens et al., 2011) and after wounding and insect attack (Zhang et al., 1996; Ohyama et al., 1998; Rosenkranz et al., 2001; Arnold and Schultz, 2002).

In our study, rapid sequestration of 11C-photosynthates belowground following wound/MeJA treatment aboveground corresponded to the induction of CWI and SOL in roots (Supplemental Fig. S8, C and F). Aboveground, young damaged leaves marginally induced CWI activity by 6 h after wound/MeJA treatment (Supplemental Fig. S8A; \( P = 0.07 \)). By 24 h, both CWI and SOL increased in all tissue types following wound/MeJA treatment (Supplemental Fig. S8).

Effect of Root Chilling on Resource Allocation

Root chilling did not alter 11CO2 fixation (Fig. 3A; \( P = 0.4815 \)) or 11C-photosynthate export (Fig. 3B; \( P = 0.2027 \)) from the LL. However, export was suppressed 2 h after wound/MeJA treatment to young leaves (Fig. 3B; \( P = 0.0012 \)). This response occurred without changes in
fixation by the LL (Fig. 3A; \( P = 0.1475 \)). In general, root chilling treatment decreased \(^{11}\text{C}\)-photosynthate allocated to YO leaves (Fig. 3, C and D; 21°C controls versus 5°C controls \( P = 0.0358 \)). Unlike plants with 21°C roots, plants with 5°C roots did not allocate more \(^{11}\text{C}\)-photosynthates to YO leaves 2 h after wound/MeJA treatment (Fig. 3, C and D; \( P = 0.4567 \)). Interestingly, plants with chilled roots also failed to allocate more \(^{11}\text{C}\)-photosynthesize to roots following wound/MeJA treatment aboveground (Fig. 4; significant effect of wound/MeJA treatment at 21°C \( P = 0.0235 \); non-significant treatment effect at 5°C \( P = 0.5712 \)).

Root chilling alone induced CWI in YO leaves and roots (Fig. 5, A and C; \( P < 0.05 \)). Under ambient root conditions, wound/MeJA treatment aboveground rapidly induced CWI and SOL activity in roots (Fig. 5, C and F; \( P < 0.0001 \)). However, in plants with roots at 5°C, wound/MeJA treatment did not alter SOL (Fig. 5F) and significantly suppressed CWI activity (Fig. 5C; \( P < 0.0001 \)) belowground. This pattern was also evident aboveground; CWI normally increased in YO leaves following wound/MeJA (Fig. 5A; \( P = 0.0075 \)). However, when roots were chilled, invertases were either no different than controls (Fig. 5A) or decreased in these leaves (Fig. 5D; \( P = 0.0019 \)).

**Effect of Root Chilling on Metabolic Partitioning of \(^{11}\text{C}\) into Soluble Sugars**

Root chilling treatment alone significantly decreased the partitioning of \(^{11}\text{C}\) into soluble sugars in the LL.
(Supplemental Fig. S9A; \( P = 0.02 \)); the accumulation of \(^{11}\text{C}\)-Suc and \(^{11}\text{C}\)-Fru was significantly suppressed (Supplemental Fig. S9, B and D; \(^{11}\text{C}\)-Suc, \( P = 0.0331 \); \(^{11}\text{C}\)-Fru, \( P = 0.0253 \)). In contrast, chilling had no effect on total or individually assayed \(^{11}\text{C}\)-sugars in YO leaves (Supplemental Fig. S10); total \(^{11}\text{C}\)-sugars increased slightly following this treatment, but means were no different from 21°C controls (Supplemental Fig. S10A; \( P = 0.161 \)). Wound/MeJA had no effect on total or individually assayed \(^{11}\text{C}\)-sugars in the LL or YO leaves of plants with chilled roots (Supplemental Figs. S9 and S10).

Effect of Root Chilling on Metabolic Partitioning of \(^{11}\text{C}\) into Organic Acids

Root chilling marginally suppressed the production of total \(^{11}\text{C}\)-phenolic acids (Supplemental Fig. S11) and \(^{11}\text{C}\)-cinnamic acid in the LL (Fig. 6A; \( P = 0.0658 \)) and YO leaves (Fig. 6B; \( P = 0.009 \)). Interestingly, the production of these compounds in the LL was suppressed even more following wound/MeJA treatment (Fig. 6A; Supplemental Fig. S11A).

DISCUSSION

Our results demonstrate that the combination of wounding and MeJA applied as a treatment to young leaves induces rapid temporal changes in the physiological and metabolic pathways in Arabidopsis as reflected by changes in allocation and metabolic partitioning of newly acquired \(^{11}\text{C}\) resources. Despite the advantages of this short-lived isotope, few studies, to our knowledge, have examined both temporal changes and the role of roots in shaping whole-plant \(^{11}\text{C}\) distribution and its use in primary and secondary metabolites following simulated herbivory.

Newly Acquired \(^{11}\text{C}\) Is Exported to Treated Young Leaves: Evidence for the Induced Sink Strength Model of Defense

Within 2 h of wound/MeJA treatment to two young leaves, we observed elevations in both \(^{11}\text{CO}_2\) fixed and \(^{11}\text{C}\)-photosynthate exported from the untreated LL.

![Figure 4](image)

**Figure 4.** Combined effect of root chilling and wound/MeJA treatment on the percentage of \(^{11}\text{C}\)-photosynthates allocated to Arabidopsis roots. All bars are mean ± se. Asterisks show significant effect of wound/MeJA treatment (\( P = 0.0235 \)). DW, Dry weight.

![Figure 5](image)

**Figure 5.** Combined effect of root chilling and wound/MeJA treatment on invertase activities in leaves and roots. Changes made by wound/MeJA treatment (black bars) are compared with representative control groups (white bars). CWI (A–C) and SOL (D–F) invertase activities in two young leaves (A and D), their respective source leaf (B and E), and root tissue (C and F) were measured at 2 h following wound/MeJA treatment. All bars are mean ± se. **\( P < 0.0001 \) and *\( P < 0.05 \) show significant effect of wound/MeJA treatment. \( n = 10 \) to 12/treatment group. FW, Fresh weight.
These findings are in agreement with studies observing increases in photosynthetic activity in unattacked leaves following damage by defoliating herbivores (Nowak and Caldwell, 1984; Welter, 1989; Schwachtje and Baldwin, 2008), a phenomenon thought to reflect a strategy to partially compensate for defoliation during herbivore attack (Detling and Painter, 1983; Nowak and Caldwell, 1984). Jasmonates are well known to decrease photosynthetic activity associated with phenylpropanoid biosynthesis (Richard et al., 2000) and the accumulation of phenolic metabolites following treatment with jasmonates and insect herbivores (Arnold et al., 2004; Hanik et al., 2010). This pattern is also consistent with previous studies demonstrating that the ability of developing leaves to respond defensively to MeJA, wounding, or insect treatment relies on their capacity to induce their sink strength for carbon-based resources and import carbon from orthostichous source leaves (Arnold and Schultz, 2002; Arnold et al., 2004).

Radioactivity present in monosaccharide sugars $^{11}$C-Glc and $^{11}$C-Fru in the LL fluctuated dramatically across the time course examined; we observed a significant decrease in these sugars from 2 to 6 h, followed by a return to baseline levels by 24 h. In our study, the 2 and 24 h time points fell at the beginning of the photoperiod (early morning), while 6 h plants were harvested in late afternoon. Thus, it is possible that the normal fluctuations in primary metabolite pools helped to accelerate the responses we observed at 2 and 24 h in young leaves. According to a recent study in *Nicotiana attenuata*, metabolites often have pronounced diurnal rhythms to their production, which may play an important role in shaping a plant’s responses to herbivore attack (Kim et al., 2011). Given these findings, and the 8-h photoperiod in which the plants for our studies were grown, it is possible that induced responses to wound/MeJA were waning by our 6 h time point, which would provide support for an underlying mechanism of circadian clock-enhanced defense in Arabidopsis (Goodspeed et al., 2012).

To our surprise, the accumulation of radiolabel in treated YO leaves at 2 h did not appear to be primarily driven by CWI or SOL invertase activity; in our time-course experiment, invertases were not significantly induced until 24 h post treatment. This result suggests that induced sink strength in young, treated leaves reflects changes in source-sink relationships followed by large-scale metabolic reprogramming associated with an increased demand for energy and carbon-based resources necessary for the production of defensive compounds. Although separating where and for what purposes additional carbon and energy are used is difficult, this alternative hypothesis is consistent with previous work in *Populus* spp., where increasing carbon export in unattacked source leaves had a positive effect on the production of secondary defense compounds in young sink leaves (Arnold and Schultz, 2002; Arnold et al., 2004).

Of the tracer remaining in aboveground sink tissues, autoradiography showed preferential allocation of $^{11}$C-photosynthate toward YO leaves rapidly (2 h) following wound/MeJA treatment. The accumulation of $^{11}$C-photosynthate in damaged YO leaves corresponded with the production of $^{11}$C-organic acids, including total $^{11}$C-phenolics and $^{11}$C-cinnamic acid, as well as the accumulation $^{11}$C-phenolics (anthocyanins) by 24 h. This is consistent with previous studies observing the induction of gene transcripts and enzyme activity associated with phenylpropanoid biosynthesis (Richard et al., 2000) and the accumulation of phenolic metabolites following treatment with jasmonates and insect herbivores (Arnold et al., 2004; Hanik et al., 2010). This pattern is also consistent with previous studies demonstrating that the ability of developing leaves to respond defensively to MeJA, wounding, or insect treatment relies on their capacity to induce their sink strength for carbon-based resources and import carbon from orthostichous source leaves (Arnold and Schultz, 2002; Arnold et al., 2004).
may also be facilitated by the push of photosynthate from source leaves, rather than these leaves locally pulling for carbon. This idea is supported by the increase in 11C-photosynthate export from the LL observed 2 h following wound/MeJA elicitation. We also found that in suc2-1 mutant plants, compromised in their ability to load photosynthate at source leaves, reactive phenolics, including anthocyanins, were not induced as dramatically in young, treated leaves, compared with their wild-type counterparts. This result provides additional support for the hypothesis that phloem-borne signals and/or resources transported from source leaves to damaged sinks is required for the induction of secondary metabolites in young leaves.

Wound/MeJA Treatment Induces Rapid Allocation of 11C-Photosynthate toward Roots

In our study, we found both allocation of newly acquired 11C-resources to wounded, young leaves and sequestration to roots to occur rapidly and simultaneously. These findings contradict our original hypothesis regarding the timeline of carbon movement following damage but are nevertheless consistent with previous work showing that enhanced movement of carbon from labeled source leaves into damaged, young leaves is coincident with transport belowground (Frost and Hunter, 2008). The rapid movement of resources belowground observed in our study is also in agreement with the growing body of literature demonstrating the transport of resources away from damaged sites and into roots and storage organs within hours following herbivory (Babst et al., 2005, 2008; Schwachtje et al., 2006; Newingham et al., 2007; Frost and Hunter, 2008; Kaplan et al., 2008; Gómez et al., 2010, 2012). The adaptive value of sequestering resources to roots has been explained in various ways, including storing reserves for later regrowth (Schwachtje et al., 2006). However, concrete evidence linking this response to tolerance remains elusive, and to date, no study exists showing the incorporation of these bunkered resources into regrown tissues.

In our study, rapid transport of 11C-photosynthate belowground corresponded to the induction of CWI and SOL invertases in roots following wound/MeJA treatment aboveground. These results suggest that carbon allocation to roots may be driven by a combination of mechanisms, including invertase-induced sink strength in the roots and increases in fixation and loading of 11C in the source leaves. By 24 h post-treatment, resource allocation to roots diminished, while allocation to the young, treated leaves remained elevated relative to controls. This pattern may reflect increases in invertase at later time points in the young leaves and/or changes made to whole-plant allocation patterns; overall, we found more radiolabel accumulating in all leaves by 24 h after treatment (see autoradiographs). It is also possible that 11C-phenolics accumulating in the LL are being transported in their intact form toward YO leaves, which may explain the increase in radiolabel in YO leaves following wound/MeJA treatment.

Root Chilling Abolishes Wound/MeJA-Induced Carbon Dynamics in Arabidopsis

To determine whether roots are a dominant sink for resources in Arabidopsis and compete with aboveground tissues for carbon resources, we used root chilling treatments to disrupt carbon flow between above- and belowground tissues. In contrast with our expectations, root chilling did not decrease root invertase activity or disrupt constitutive whole-plant allocation patterns of 11C-photosynthate. In fact, root chilling significantly induced invertase activity in roots. Despite the fact that we observed no changes in newly acquired carbon allocation patterns following root chilling, it is still possible that this treatment was capable of influencing turnover in 12C-sugars pools in roots (e.g. by increasing root exudation) to drive the observed changes in invertases.

Compared with plants with roots at ambient temperature, plants with chilled roots failed to allocate newly acquired carbon resources belowground or transport carbon toward wounded YO leaves following wound/MeJA. The increase in 11CO2 fixed and in 11C-photosynthate exported by the LL following wound/MeJA treatment was also eliminated when roots were chilled, suggesting that roots may play an integral role in controlling how plants respond defensively aboveground in Arabidopsis. Placement of plants under low temperature conditions is known to trigger the biosynthesis of the plant hormone abscisic acid, which not only activates genes induced by drought, salt, and cold stress, but is also known to down-regulate genes related to defense (Anderson et al., 2004). In Arabidopsis, Suc synthesis genes, including those encoding Suc phosphate synthase, are induced by low temperature (Usadel et al., 2008), while transcript levels of several members of the invertase family, as well as overall levels of invertase activity, are suppressed. In contrast, other plant species (e.g. wheat [Triticum aestivum], potato [Solanum tuberosum], and tomato [Solanum lycopersicum]) exhibit the up-regulation of invertase following decreases in temperature (Artuso et al., 2000; Vargas et al., 2007; Janská et al., 2010). Together, these findings underline a key role for carbohydrate metabolism under low temperature conditions (Kaplan et al., 2007; Guy et al., 2008). In our study, while 11C-photosynthate appeared to accumulate in excess in the LL following root chilling treatment, this accumulation did not correspond to increases in any of the primary or secondary metabolites measured. This finding suggests that newly acquired carbon may be partitioned to other functions such as long-term storage pools (starch) or carbon skeletons for compounds such as amino acids.
Resolving the Bunkering Conundrum

This work reveals that many induced plant defense responses are rapid, complex, and transient in nature. Our results indicate that Arabidopsis simultaneously transports newly acquired carbon belowground even as it increases transport toward damaged sites. These responses likely reflect mechanisms to tolerate herbivory and to up-regulate secondary defense chemistry at sites of attack, both of which may be driven in part by increased fixation and export by source leaves and systemic induction of invertases. Whole-plant tracer dynamics corresponded to the local induction of phenolic compounds in the plant’s defense chemistry production. However, when root chilling treatments were combined with wound/MelA elicitation, aboveground tissues were unresponsive, suggesting that roots play a crucial role in shaping how plants respond defensively aboveground through an integrated communication network.

Growing root apices are well known to sense changes in their local abiotic and biotic environments and respond to them accordingly with changes to growth (Monshausen and Gilroy, 2009). Therefore it is possible that roots may direct the signaling that causes a rapid shuffling of resources to roots following herbivory and subsequent return to leaves to cue systemic defenses aboveground at later time points. Current evidence in maize (Ze a mays) and tobacco (Nicotiana tabacum), as well as studies using Arabidopsis, suggest that roots are capable of synthesizing many secondary metabolites, serve as dynamic storage organs, and may also relay information regarding changes to their abiotic and biotic environment in a shoot-root-shoot loop in plant defense systems (Erb et al., 2009). Thus, future studies using short-lived isotopes with Arabidopsis may elucidate shoot-root and root-shoot signals that underlie carbon resource allocation and metabolic partitioning to plant defense chemistry to provide a better understanding of source-sink interactions in relation to plant defenses and their importance for pest resistance in other plant species.

MATERIALS AND METHODS

Plant Material

For 13C-labeled studies, Arabidopsis (Arabidopsis thaliana; Columbia-0) seeds were germinated on Rockwool cylinders soaked in Hoagland solution (Hoagland modified basal salt mixture; Phyto Technology Laboratories) at 21°C and 62% relative humidity with an 8/16-h (180 mol m−2 s−1) photoperiod. Two-week-old seedlings were transplanted into individual pots containing a 4:1 mixture of sand:zeolite (Sakrete multipurpose sand, Sakrete of North America; Zeopro zeolite mix, Zeoponix). Zeolite was soaked in Hoagland solution to wet, then mixed by hand with sand. For large-scale experiments measuring 13C-phenolics and invertase, Arabidopsis plants were germinated on agar plates containing Murashige and Skoog media. Homozygote seedlings for experiments by their characteristic phenotype following germination on media without Suc; mutants are visibly smaller than the wild type, with very short primary roots and translucent cotyledons (Gottwald et al., 2000). Approximately 2 weeks post germination, homozygous plants were rescued on fresh media containing 1% supplemental Suc. Plants that were successfully rescued were transplanted to pots containing Metro-Mix 200 soil (Sun Gro Horticulture) supplemented with 1.8 kg of Osmocote slow-release fertilizer (The Scotts Company) per cubic meter of soil. Plants were maintained under the same growth conditions described above. In all experiments, plants were approximately 4 weeks postgermination and contained 21 ± 2 leaves.

Treatments and Experimental Design

MeJA is a well-known plant defense elicitor that up-regulates the production of defensive compounds against herbivores and is often used to mimic the effect of herbivory (Gundlach et al., 1992; Mc Conn et al., 1997; Baldwin and Hamilton, 2000). Young leaves are frequently preferred by herbivores as a result of their higher nutrient content and reduced toughness (McKey, 1974; Coley et al., 2006). Thus, we simulated herbivore damage on two young leaves (Supplemental Fig. S12, shown in purple) sharing direct vascular connections (orthostichous) to a mature source leaf (approximately ninth leaf; yellow in Supplemental Fig. S12) by damaging leaves perpendicularly to the midvein with a pattern wheel and applying 20 μL of an aqueous dilution of MeJA (115 μM; Sigma-Aldrich) to each leaf. Leaves were assigned numbers by counting down from the apex, with leaf 1 designated as the youngest leaf that was at least 5 mm in length.

To capture temporal changes in resource allocation in response to simulated herbivory, the LL was pulse labeled with 13CO2 at 1, 5, and 23 h following wound and MeJA treatment. Plants were allowed to incubate with the tracer for 1 h, during which 13C-uptake and export by the LI were measured. Partitioning of 13C-photosynthate into primary and secondary metabolites was measured in the LL and two YO leaves immediately following this incubation period.

During the labeling procedure, the emission of radioactivity by the 13C-labeled plants required special containment, limiting analysis to a single plant per time point. Labeling experiments were repeated to average approximately six plants per time point (2, 6, and 24 h). Control plants were left untreated but were placed in the tracer administration box during the time course. The time-course experiment was repeated in the absence of 13CO2 labeling to assess changes in CWI and SOL invertase activity as well the accumulation 13C-phenolics.

suc2-1 Study

Arabidopsis loads phloem from source leaves into the companion cell/sieve element (CC/SE) complex apoplastically (i.e. CC/SE complexes are isolated from the symplast of the surrounding cells by a shortage of plasmodesmatal connections; Haritatos et al., 2000). As a result, photosynthate and presumably other phloem-transported metabolites must first be exported from mesophyll cells into the apoplast and then loaded into the CC/SE complex by an energy-dependent transport system (DeWitt and Sussman, 1995). In Arabidopsis, this process is primarily driven by Suc transporters (Stadler and Sauer, 1996). Source leaves of the Arabidopsis T-DNA insertion line in AtSUC2 (suc2-1), a phloem-specific Suc transporter gene (Truernit and Sauer, 1995; Stadler and Sauer, 1996), contain excess starch and fail to transport radiolabeled sugar efficiently to roots and inflorescences (Gottwald et al., 2000). This mutant not only provides strong evidence in support of apoplastic loading as the primary method for initiating long-distance transport in Arabidopsis, but offers a unique opportunity to test whether phloem loading at the source and subsequent transport to sink tissues is required for the induction of secondary chemistry.

Mutant seeds (The Arabidopsis Information Resource) were grown side by side with wild-type (Wassilewskija-2) and heterozygous siblings for controlled comparisons. Seeds were surface sterilized and germinated on agar plants containing Murashige and Skoog media. Homozygote seedlings were screened for experiments by their characteristic phenotype following germination on media without Suc; mutants are visibly smaller than the wild type, with very short primary roots and translucent cotyledons (Gottwald et al., 2000). Mutants were rescued on fresh media containing 1% supplemental Suc. Plants that were successfully rescued were transplanted to pots containing Metro-Mix 200 soil (Sun Gro Horticulture) supplemented with 1.8 kg of Osmocote slow-release fertilizer (The Scotts Company) per cubic meter of soil. Homozygous and wild-type plants were approximately 6 weeks post germination at the time of the experiment. Homozygous plants were generally slower to develop than their wild-type siblings and so were smaller at the time of the experiment (contained approximately four less leaves).

We used a factorial design comprised of 32 plants randomly assigned to one of the following treatments: (1) control, wild type; (2) wound/MelA, wild type; (3) control, suc2-1; and (4) wound/MelA, suc2-1. As in the labeling studies, MelA treatments were applied to sink leaves 1 and 4. Damaged sink leaves and leaves from the apex of the rosette were harvested in liquid nitrogen 48 h later for phenolic measurements.

700 Plant Physiol. Vol. 161, 2013
Root Chilling Treatment

In Arabidopsis, roots are normally a significant sink, drawing resources to a greater extent than aboveground tissues (Ferreiri et al., 2012). To determine whether roots effectively compete with defense-induced primary and secondary metabolites for carbon resources, root chilling treatments were used to disrupt carbon flow between above- and belowground tissues. The inhibition of phloem transport by chilling roots has been particularly well investigated (Geiger, 1969; Minchin et al., 1983, 1994; Poiré et al., 2010; Thorpe et al., 2010) and can be accomplished with minor drops in temperature (Minchin and Thorpe, 1983). In our experiments, individual plants were seated inside an epoxy-sealed pot affixed in an ice water bath (°C; Supplemental Fig. S13A). Plants incubated for 1 h (the time needed for soil to reach 5°C; Supplemental Fig. S13B) before wound/MeJA treatments occurred and remained in the ice bath during the 1-h incubation following treatment as well as for the 1-h incubation with the tracer. Control plants were left untreated but were placed in the root chilling station for the same duration as wound/MeJA-treated plants. The experiment was repeated at a larger scale (root chilling array; Supplemental Fig. S13C) to assess changes in CWI and SOL invertase activities as well as total 13C-phenolics in two young developing leaves, their source leaf, and roots.

Radiotracer Production

13C, a short-lived radioisotope (t1/2 = 20.4 min) was used to examine the uptake, transport, and allocation of 13C-photosynthates. 13C was made by irradiating a nitrogen gas (N2) target with 17-MeV protons from the TR-19 cyclotron (Ebloco Industries) at Brookhaven National Laboratory to induce the 13N(n,α)13C nuclear transformation (Ferrieri and Wolf, 1983).

Radiotracer Administration

Carbon dioxide labeled with 13C was captured on a molecular sieve (A4), desorbed, and quickly released into an air stream at 200 mL min−1 as a discrete pulse to the targeted LL (leaf 9) fixed inside a 5 × 10-cm airtight cell maintained at 21°C and fitted with red/blue light-emitting diodes (290 nm−1 s−1) to ensure a steady level of fixation (Supplemental Fig. S14A). The LL was pulse fed 13CO2 for 1 min, then chased with normal air for the duration of the 1-h incubation with the tracer. A PIN diode radiation detector (Carroll Ramsey Associates) affixed to the bottom of the leaf cell enabled continuous measurement of radioactivity levels within the cell.

Measuring Whole-Plant Carbon Dynamics

13CO2 fixation and leaf export of 13C-photosynthate were measured for each plant and compared with controls. The difference between the amount of radioactivity registered in the pulse height and residual radioactivity after the pulse had passed through the cell was a reflection of leaf tracer fixation (Supplemental Fig. S14A). Export of the radiotracer from the LL was calculated as the percentage of activity that left the labeled leaf after 1 h (Supplemental Fig. S14B). 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. Leaves and roots were placed into separate scintillation vials and counted using a γ counter (Picker). All radioactivity measurements were decay corrected to a standard zero time of each study to quantify allocation of 13C-photosynthate above- and belowground.

Positron Autoradiography

To determine the distribution of 13C-photosynthates throughout aboveground tissues, we used phosphor plate imaging of positron emissions (Fuji 2500 imager). One hour after pulse labeling of the targeted LL, treated and untreated rosette leaves (not including the LL) were excised at the soil line and exposed for 15 min on a phosphor imaging plate (Fuji BAS2500). The remaining LL petiole, YO leaves (wound/MeJA or corresponding control leaves of same leaf number) and untreated rosette leaves were cut away from the remaining LL petiole, YO leaves (wound/MeJA or corresponding control leaves), YO leaves (wound/MeJA or corresponding control leaves) and untreated rosette leaves (not including the LL) were excised at the soil line and incorporated into autoradiographs.

Tissue Extraction and Analysis of Radioactive Soluble Sugars

One hour after tracer administration, the portion of LL tissue enclosed in the cell (exposed to 13CO2) was cut away from the remaining leaf, weighed, and flash frozen in a microtube immersed in liquid nitrogen. Frozen tissue was ground to a fine powder using a pelleting tissue grinder (Kontes disposable pellet pestles; 1.5 mL) and extracted in 1 mL of acidified methanol (1% v/v HCl in methanol) for 10 min at 90°C. The extract was separated by pipette and filtered through a 0.22-μm filter (Millipore). Immediately following autoradiography of aboveground tissues, two treated YO leaves (or equivalent controls) were weighed together and subjected to the same extraction procedure.

13C-soluble sugars were separated from final extracts by thin-layer chromatography by spotting 2 μl onto silica NH2 HP TLC plates (Sorbert Technologies; B. Babst, personal communication). Plates were developed in 4:1 acetonitrile-water, air-dried, and exposed for 30 min on a phosphor imaging plate (Fuji BAS2500). The incorporation of 13C into soluble sugars was quantified using ImageGauge software and related to total extract radioactivity determined by γ counting (Picker). Individual soluble sugars (Suc, Glc, Fru, raffinose, and maltoose) were identified by comparing retention factor values of sugars from sample extracts to those of authentic 13C-sugar standards. 13C-sugars were visualized colorimetrically by dipping plates into a derivatization reagent of 5% 1-napthyl in concentrated H2SO4.

Analysis of 13C-Organic Acids

Following 13C-soluble sugar analysis, remaining acidified methanol extracts were hydrolyzed by adding 2 μL KOH (6 N) and heating for 10 min at 90°C. Extracts were transferred by glass pipette to NH4 sep-pak cartridges (Waters) and washed with 2 mL each of water, isopropyl alcohol, acid acetate (1:1 v/v), ether, and anionitrite. Elute from each wash was collected and counted using a γ counter (Picker). Phenolic acids were eluted in 2 mL 2% formic acid in methanol, heated to dryness, and reconstituted in 50 μL methanol.

The extent to which 13C-phosynthates were incorporated into Arabidopsis organic acids was determined in 5 μL of this final methanol extract using two-dimensional thin-layer chromatography (Chapple et al., 1992). Silica NH2 HP TLC plates (Sorbert Technologies) were run first in petroleum ether:ethyl acetate:methanol:acetic acid (10:10:1:0.2) and then in toluene: glacial acetic acid (2:1) saturated with water. Plates were air-dried and immediately exposed for a minimum of 3 h on phosphor plates. The percentage of radioactivity present in 13C-organic acid compounds in each extract was quantified using ImageGauge software and related to total extract radioactivity.

13C-labeled organic acids (total phenolics and cinnamic acid) were identified by comparing retention factor values to those of phenolics commonly found in Arabidopsis. 13C-phenolic standards were visualized under long-wave UV light (365 nm) by their characteristic fluorescence under short-wave UV light (254 nm) by their characteristic absorbance (Chapple et al., 1992).

13C-Phenolics Extraction and Quantification

Individual leaves and roots were freeze-dried (4 to 6 mg dry weight) and ground in a Tafelboys high-throughput homogenizer (Tromemher). Phenolics were extracted overnight in 200 μL of 1% (v/v) HCl in methanol at 4°C. An additional extraction with 250 μL distilled water and 500 μL of chloroform was used to remove chlorophyll from all samples (Choi et al., 2009). Samples were vortexed and centrifuged for 3 min at 3000g. Relative anthercyanin levels in the aqueous phase were determined spectrophotometrically by measuring A530. Total flavonoid compounds were also estimated in the same extracts at absorbance 320 nm (Fukumoto and Mazzza, 2000; Shao et al., 2008). The concentration of total reactive phenolics present in leaf extracts was determined using the Folin-Denis assay. Standard curves were developed using chlorogenic and gallic acids as well as standards purified from each treatment group (Appel et al., 2001).

Invertase Activity Assay

The activity of CWI and SOL invertases were measured in leaves and roots according to previously described methods (Arnold and Schultz, 2002).
Briefly, proteins were extracted from fresh frozen and ground tissue in 6 volumes (volume g fresh weight$^{-1}$) of MES buffer (pH 7.0) containing 5 mM EDTA, 5% (w/v) polyvinylpolypyrrolidone. Ser proteinase inhibitor DTT (20 mM), and benzamidene (2.5 mM). Samples were sonicated on ice for 30 min and SOL fractions were transferred to new tubes. Remaining pellets (containing CWI) were washed three times with deionized water. CWI and SOL fractions were suspended in assay buffer (0.05 M acetic acid, 0.05 M MES, 0.01 M Trizma, and 3 mM sodium azide, pH 4.5) containing 200 mM Suc. Suc cleaved by CWI and SOL fractions was assayed by the generation of Glc monomers during a 15-min incubation period (pH 4.5, 37°C) and quantified (μM Suc cleaved g fresh weight$^{-1}$ min$^{-1}$) colorimetrically by their $A_{540}$.

**Statistical Analyses**

One-way and two-way ANOVAs were performed, followed by Tukey honestly significant difference post-hoc comparisons. Normality and equality of variance were verified using Kolmogorov-Smirnov and Levene's tests, respectively. All data were analyzed using SAS statistical package version 9.3 (SAS Institute).

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Percentage of total $^{13}$C-photosynthates allocated to Arabidopsis roots.

**Supplemental Figure S2.** $^{13}$C-soluble sugar profiles in the load leaf.

**Supplemental Figure S3.** $^{13}$C-soluble sugar profiles in two young treated leaves.

**Supplemental Figure S4.** Partitioning of recently fixed $^{13}$CO$_2$ into total phenolic acids.

**Supplemental Figure S5.** Partitioning of recently fixed $^{13}$CO$_2$ into cinnamic acid.

**Supplemental Figure S6.** Effect of wound/MeJA treatment on Folin-Denis reactive phenolic compounds.

**Supplemental Figure S7.** Effect of wound/MeJA treatment on anthocyanin accumulation in two young treated leaves and systemic leaves of suc2-1 and wild-type (Ws-2) plants.

**Supplemental Figure S8.** Effect of wound/MeJA treatment on invertase activities in leaves and roots.

**Supplemental Figure S9.** Combined effect of root chilling and wound/MeJA treatments on $^{13}$C-soluble sugars in the load leaf.

**Supplemental Figure S10.** Combined effect of root chilling and wound/MeJA treatments on $^{13}$C-soluble sugars in two young treated leaves.

**Supplemental Figure S11.** Combined effect of root chilling and wound/MeJA treatments on the partitioning of recently fixed $^{13}$CO$_2$ into total phenolic acids.

**Supplemental Figure S12.** Arabidopsis plant illustrating the load leaf and YO, treated leaves.

**Supplemental Figure S13.** Experimental setup for root chilling studies.

**Supplemental Figure S14.** Experimental setup for pulse labeling with $^{13}$CO$_2$.

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**LITERATURE CITED**

Anderson JP, Badrzasauferi E, Schenk PM, Manners JM, Desmond OJ, Ehler C, Melean DC, Ebert PR, Kazan K (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell 16: 3460–3479.

Appel HM, Govener HL, D’Ascenzo M, Siska E, Schultz JC (2001) Limitations of folin assays of foliar phenolics in ecological studies. J Chem Ecol 27: 761–778.

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 Phytol 164: 157–164.

Arnold T, Schultz J (2002) Induced sink strength as a prerequisite for induced tannin biosynthesis in developing leaves of Populus. Oecologia 130: 585–593.

Artuso A, Guidi L, Soldatini GF, Pardossi A, Tognoni F (2000) The influence of chilling on photosynthesis and activities of some enzymes of sucrose metabolism in Lycopersicon esculentum Mill. Acta Physiol Plant 22: 193–201.

Babst R, Ferri et al. (2008) Lymnantria dispar herbivory induces rapid changes in carbon transport and partitioning in Populus nigra. Entomol Exp Appl 128: 117–125.

Babst BA, Ferri et al. RA, Gray DW, Lerdau M, Schlyer DJ, Schueler M, Thorpe MR, ORIANS CM (2005) Jasmonic acid induces rapid changes in carbon transport and partitioning in Populus. New Phytol 167: 63–72.

Baldwin I, Hamilton W (2000) Jasmonate-induced responses of Nicotiana sylvestris results in fitness costs due to impaired competitive ability for nitrogen. J Chem Ecol 26: 915–952.

Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc Natl Acad Sci USA 95: 8113–8118.

Barrett DH, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ, Smith AM (2009) Normal growth of Arabidopsis requires cytosolic invertase but not sucrose synthase. Proc Natl Acad Sci USA 106: 13124–13129.

Barton KE, Kocicheva J (2010) The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am Nat 175: 481–493.

Bazzaz F, Chiariello N, Coley P, Pitelka L (1987) Allocating resources to reproduction and defense. Bioscience 37: 58–67.

Beltranj R, Ronco M, Montaldi E, Carbone A (1998) Senescence of flag leaves and ears of wheat hastened by methyl jasmonate. J Plant Growth Regul 17: 53–57.

Chapple CCS, Vogt T, Ellis BE, Somerville CR (1992) An Arabidopsis mutant defective in the general phenylpropanoid pathway. Plant Cell 4: 1413–1424.

Choi S, Kwon Y, Hossain M (2009) A mutation in ELA1, an age-dependent negative regulator of PAPI/MYB75, causes UV- and cold stress-tolerance in Arabidopsis thaliana seedlings. Plant Sci 176: 678–686.

Coley PD, Bateman ML, Kursar TA (2006) The effects of plant quality on caterpillar growth and defense against natural enemies. Oikos 115: 219–228.

Creelman RA, Muller JF (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48: 355–381.

Delling J, Painter E (1983) Defoliation responses of western wheatgrass populations with diverse histories of prairie dog grazing. Oecologia 57: 65–71.

DeWitt ND, Sussman MR (1995) Immunocytochemical localization of an epitope-tagged plasma membrane proton pump (H$^+$-ATPase) in phloem companion cells. Plant Cell 7: 2053–2067.

Erb M, Lenk C, Degenhardt J, Turlings TCJ (2009) The underestimated role of roots in defense against leaf attackers. Trends Plant Sci 14: 653–659.

Ferri et al. AP, Appel H, Ferri et al. RA, Schultz JC (2012) Novel application of 2-$^{18}$Ffluro-2-deoxy-glucose to study plant defenses. Nucl Med Biol 39: 1152–1160.

Ferri et al. R, Gray D, Babst B, Schueler M, Schlyer D, Thorpe M, ORIANS C, Lerdau M (2005) Use of Carbon-11 in Populus shows that exogenous jasmonic acid increases biosynthesis of isoprene from recently fixed carbon. Plant Cell Environ 28: 591–602.

Ferri et al. R, Wolf A (1983) The chemistry of positron emitting nucleogenic atoms with regard to preparation of labeled compounds of practical utility. Radiocim Acta 34: 69–83.
Frost CJ, Hunter MD (2008) Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. New Phytol 178: 835–845

Fukumoto LR, Mazza G (2000) Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem 48: 3597–3604

Geiger D (1969) Chilling and translocation inhibition. Ohio J Sci 69: 356–366

Gómez S, Ferrieri RA, Schueller M, Orians CM (2010) Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. New Phytol 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. Entomol Exp Appl 144: 101–111

Gooldspeed D, Chehab EW, Min-Venditti A, Braam J, Covington MF (2012) Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. Proc Natl Acad Sci USA 109: 4674–4677

Gottwald JR, Krysan PJ, Young JC, Evert RF, Sussman MR (2000) Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proc Natl Acad Sci USA 97: 13979–13984

Gundlach H, Müller MJ, Kutchan TM, Zenk MH (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci USA 89: 2390–2393

Guy C, Kaplan F, Kopka J, Selbig J, Hincha DK (2008) Metabolomics of temperature stress. Physiol Plant 132: 220–235

Hanáčký S, Senn J (1992) Sources of variation in rapidly inducible responses to leaf damage in the mountain birch-insect herbivory system. Oecologia 91: 318–331

Hanik N, Gómez S, Best M, Schueller M, Orians CM, Ferrieri RA (2010) Partitioning of new carbon as 14C in Nicotiana tabacum reveals insight into methyl jasmonate induced changes in metabolism. J Chem Ecol 36: 1058–1067

Haritatos E, Medville R, Turgeon R (2001) Jasmonate-inducible gene: What does it mean? Trends Plant Sci 14: 87–91

Heger A, Schieder H, Thorpe MR, Kuhn AJ, Schurr U, Walter A (2010) Root cooling strongly affects die leaf growth dynamics, water and carbohydrate relations in Ricinus communis. Plant Cell Environ 33: 408–417

Rehill BJ, Schultz JC (2003) Enhanced invertase activities in the galls of Hymenaphis hammelidellus. J Chem Ecol 29: 2703–2720

Richard S, Lapointe G, Rutledge RG, Séguin A (2000) Induction of chalcone synthase expression in white spruce by wounding and jasmonate. Plant Cell Physiol 41: 982–987

Roitsch T, González MC (2004) Function and regulation of plant invertases: sweet sensations. Trends Plant Sci 9: 606–613

Rosenkrantz H, Vogel R, Greiner S, Rausch T (2001) In wounded sugar beet (Beta vulgaris L.) tap-root, hexose accumulation correlates with the induction of a vacuolar invertase isoform. J Exp Bot 52: 2381–2385

Schwachtje J, Baldwin IT (2008) Why does herbivore attack reconfigure primary metabolism? Plant Physiol 146: 845–851

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. Proc Natl Acad Sci USA 103: 12935–12940

Shao L, Zhu P, Peng C, Lin Z, Yang C, Gu Q (2008) Enhanced sensitivity of Arabidopsis anthocyanin mutants to photooxidation: a study with fluoroescence imaging. Funct Plant Biol 35: 714–723

Siemens J, González MC, Wolf S, Hofmann C, Greiner S, Du Y, Rausch T, Roitsch T, Ludwig-Müller J (2011) Extracellular invertase is involved in the regulation of clubroot disease in Arabidopsis thaliana. Mol Pathol 12: 247–262

Stadler R, Sauer N (1996) The Arabidopsis thaliana AtSUC2 gene is specifically expressed in companion cells. Bot Acta 109: 299–306

Tang GQ, Lüscher M, Sturm A (2001) The promoter of the AtSUC2 gene is specific for sucrose transporters. Plant Physiol 126: 4117–4127

Mithöfer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63: 431–450

Monshausen GB, Gilny S (2009) The exploring root: root growth responses to local environmental conditions. Curr Opin Plant Biol 12: 766–772

Newingham RA, Callaway RM, Bassirirad H (2007) Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory. Oecologia 153: 913–920

Nowak R, Caldwell M (1984) A test of compensatory photosynthesis in the field: implications for herbivory tolerance. Oecologia 61: 311–318

Ohyama A, Nishimura S, Hirai M (1998) Cloning of cDNA for a cell wall-bound invertase from tomato (Lycopersicon esculentum) and expression of soluble and cell wall-bound invertases in plants and wounded leaves of L. esculentum and L. peruvianum. Genes Genet Syst 73: 149–157

Orians C, Jones C (2001) Plants as resource mosaics: a functional model for predicting patterns of plant–resource heterogeneity to consumers based on vascular architecture and local environmental variability. Oikos 94: 493–504

Orians CM, Thorn A, Gómez S (2011) Herbivore-induced resource sequestration in plants: Why bother? Oecologia 167: 1–9

Pawels L, Inzé D, Goossens A (2009) Jasmonate-inducible gene: What does it mean? Trends Plant Sci 14: 87–91

Phillips R, Schneider H, Thorpe MR, Kuhn AJ, Schurr U, Walter A (2010) Root cooling strongly affects die leaf growth dynamics, water and carbohydrate relations in Ricinus communis. Plant Cell Environ 33: 408–417

Richard S, Lapointe G, Rutledge RG, Séguin A (2000) Induction of chalcone synthase expression in white spruce by wounding and jasmonate. Plant Cell Physiol 41: 982–987

Roitsch T, González MC (2004) Function and regulation of plant invertases: sweet sensations. Trends Plant Sci 9: 606–613

Rosenkrantz H, Vogel R, Greiner S, Rausch T (2001) In wounded sugar beet (Beta vulgaris L.) tap-root, hexose accumulation correlates with the induction of a vacuolar invertase isoform. J Exp Bot 52: 2381–2385

Schwachtje J, Baldwin IT (2008) Why does herbivore attack reconfigure primary metabolism? Plant Physiol 146: 845–851

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. Proc Natl Acad Sci USA 103: 12935–12940

Shao L, Zhu P, Peng C, Lin Z, Yang C, Gu Q (2008) Enhanced sensitivity of Arabidopsis anthocyanin mutants to photooxidation: a study with fluoroescence imaging. Funct Plant Biol 35: 714–723

Siemens J, González MC, Wolf S, Hofmann C, Greiner S, Du Y, Rausch T, Roitsch T, Ludwig-Müller J (2011) Extracellular invertase is involved in the regulation of clubroot disease in Arabidopsis thaliana. Mol Pathol 12: 247–262

Stadler R, Sauer N (1996) The Arabidopsis thaliana AtSUC2 gene is specifically expressed in companion cells. Bot Acta 109: 299–306

Tang GQ, Lüscher M, Sturm A (2001) The promoter of the AtSUC2 gene is specific for sucrose transporters. Plant Physiol 126: 4117–4127

Thorpe MR, Ferrieri AP, Herth MM, Ferrieri RA (2007) 13C-imaging: Methyl jasmonate moves in both phloem and xylem, promotes transport of jasmonate, and of photoassimilate even after proton transport is decoupled. Planta 226: 541–551

Thorpe MR, Furch AC, Minchin PE, Fölljer J, Van Bel AJ, Hafke JB (2010) Rapid cooling triggers forisome dispersion just before phloem transport stops. Plant Cell Environ 33: 259–271

Truernit E, Sauer N (1995) The promoter of the Arabidopsis thaliana SUC2 gene is specifically expressed in companion cells. Bot Acta 109: 299–306

Usadel B, Bläsiing OE, Gibon Y, Poree F, Höhne M, Günter M, Trethewey R, Kamlange B, Poetter H, Stitt M (2008) Multilevel genomic analysis of the response of transcripts, enzyme activities and metabolites in Arabidopsis rosettes to a progressive decrease of temperature in the non-freezing range. Plant Cell Environ 31: 518–547

Vargas WA, Pontis HG, Salerno GL (2007) Differential expression of alkaline and neutral invertases in response to environmental stresses: Plant Physiol. Vol. 161, 2013
characterization of an alkaline isoform as a stress-response enzyme in wheat leaves. Planta 226: 1535–1545

Weller SC (1989) Arthropod impact on plant gas exchange. In EA Bernays, ed, Insect-Plant Interactions, Vol 1. CRC Press, Boca Raton, FL, pp 135–150

Weschke W, Panitz R, Gubatz S, Wang Q, Radchuk R, Weber H, Wobus U (2003) The role of invertases and hexose transporters in controlling sugar ratios in maternal and filial tissues of barley caryopses during early development. Plant J 33: 395–411

Wool D (2004) Gallng aphids: specialization, biological complexity, and variation. Annu Rev Entomol 49: 175–192

Zangerl A, Bazzaz F (1992) Theory and pattern in plant defense allocation. In RS Frizz, EL Simms, eds, Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics. University of Chicago Press, Chicago, pp 363–391

Zhang L, Cohn NS, Mitchell JP (1996) Induction of a pea cell-wall invertase gene by wounding and its localized expression in phloem. Plant Physiol 112: 1111–1117