Spatial and Temporal Dynamics of Jasmonate Synthesis and Accumulation in Arabidopsis in Response to Wounding*

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A new metabolite profiling approach combined with an ultra-rapid sample preparation procedure was used to study the temporal and spatial dynamics of the wound-induced accumulation of jasmonic acid (JA) and its oxygenated derivatives in Arabidopsis thaliana. In addition to well known jasmonates, including hydroxyjasmonates (HOJAs), jasmonoyl-isoleucine (JA-Ile), and its 12-hydroxy derivative (12-HOJA-Ile), a new wound-induced dicaresyljasmonate, 12-carboxyjasmonoyl-L-isoleucine (12-HOOCJA-Ile) was discovered. HOJAs and 12-HOOCJA-Ile were enriched in the midveins of wounded leaves, strongly differentiating them from the other jasmonate metabolites studied. The polarity of these oxylipins at physiological pH correlated with their appearance in midveins. When the time points of accumulation of different jasmonates were determined, JA levels were found to increase within 2–5 min of wounding. Remarkably, these changes occurred throughout the plant and were not restricted to wounded leaves. The speed of the stimulus leading to JA accumulation in leaves distal to a wound is at least 3 cm/min. The data give new insights into the spatial and temporal accumulation of jasmonates and have implications in the understanding of long-distance wound signaling in plants.

Jasmonic acid (JA) is a potent lipid-derived regulator that plays diverse and complex roles in protecting plants from herbivores and some pathogens (1–3), in growth control in wounded plants (4), and in reproduction (5, 6). Among the best characterized roles of JA and its metabolites are its actions in wounded tissues. Jasmonates control, directly or indirectly, the levels of an estimated 67–84% of herbivore-inducible transcripts (7). JA, or a related molecule may move and amplify jasmonate production in vascular tissues (8). In the form of its methyl ester, JA may be transported over long distances within the plant to mediate systemic wound signaling (9). Much of the JA synthesis occurring in response to attack seems to be associated with the vascular system (2). In response to wounding, levels of the molecule in the wounded leaf greatly exceed levels in resting leaves, increasing up to at least 40-fold within 90 min after a single wound (10). After 90 min, JA levels start to subside, reaching half-maximal values ~9 h after wounding (11). Given the potent signaling activity of certain derivatives of JA (12), a full elucidation of its turnover is essential for understanding how jasmonate levels are brought back toward resting levels subsequent to wounding.

The biologically active jasmonic acid (＋)-(3R,7S)-JA (cis configuration of the cyclopentanone ring) can be inactivated by epimerization in vivo to (−)-(3R,7R)-JA (trans) (13), providing a first line of deactivation of the signal. However, because this epimerization is reversible and at equilibrium some (＋)-(3R,7S)-JA remains, JA must be cleared from the cell by other mechanisms. Over 10 metabolites resulting from JA modification have been detected in vivo (14). Modified JAs include biologically active amido adducts with amino acids such as, jasmonoyl-isoleucine (JA-Ile). A JA-Ile derivative, hydroxylated on carbon 12 of the jasmonoyl moiety, was recently discovered (15). Another set of modified JAs includes hydroxy JAs such as 12-hydroxyjasmonate and its sulfonate derivative (16) and JA glucosides (17). cis-Jasmonate, not to our knowledge reported from Arabidopsis, may be an inactivated version of JA destined for clearance from the cell (18).

What is currently unclear for leaves is the spatial and temporal dynamics of the production of JA and its derivatives. Where and when are JA derivatives made? In which tissues or organs do they accumulate? Does this correlate with their physico-chemical properties? A non-targeted metabolomic approach was used to study changes in metabolite profiles that occurred in response to wounding. The production of biologically active jasmonates occurred within a time frame clearly distinguishable from the production of oxygenated JA metabolites. To see where various jasmonates accumulated, midveins were dissected out of leaves and their jasmonate content compared with the jasmonate content of the leaf. Several polar derivatives of JA and JA-Ile were detected, including a novel highly oxygenated molecule. Accumulation in the midveins of the plants correlated with the polarity of the molecules. During the course of these experiments we noted apparent differences in the level of JA in resting leaves according to the protocol employed. Using ultrarapid harvesting and extraction, we found that systemic JA...
accumulation in response to a wound was far more rapid than previously assumed.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Isopropanol was obtained from SDS (Peypin, France). Liquid chromatography mass spectrometry-grade acetonitrile and water from Fisher Scientific (Loughborough, UK) were used for the ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOF-MS) analyses. For semi-preparative isolation, solvents were HPLC-grade acetonitrile Chromanorm from VWR (Leuven, Belgium) and deionized water filtered on 0.22-μm membrane filters from Millipore AG. For capillary NMR (CapNMR) experiments, methanol-d₄ (CD₃OD; 99.8% atom deuterium) was obtained from Armar Chemicals (Buchs, Switzerland).

**Plant Growth, Wounding Experiments—**Arabidopsis thaliana accession Columbia-0 (Col-0), the coi1–1 mutant (19), and the jasmonate response mutant opr3 in the Wassilewskija background (20) were grown at 22 °C, 70% relative humidity, under the jasmonate response mutant was wounded and harvested after 90 min. For quantitative experiments in Fig. 4, the Weber et al. (10) protocol was used. In separate experiments, local and distal central midveins were rapidly removed with a scalpel. Wounded leaves were also harvested at several short time points (5, 15, 45 min). The opr3 mutant was wounded and harvested after 90 min. For quantitative experiments in Fig. 4A, the Weber et al. (10) protocol was used. In these experiments Col-0 and the coi1–1 mutant were wounded, weighed, and harvested after 5, 15, 30, and 90 min.

**Extraction Procedure and UPLC-TOF-MS Experiments**—A very rapid procedure (2 min) was used for the extraction using a ball mill extractor Retsch MM200, Schieritz & Hauenstein AG (Arlesheim, Switzerland). Isopropanol was employed as a solvent for the extractions prior to UPLC-TOF-MS analyses and semi-preparative chromatography. Crude extracts were evaporated to dryness and weighed. A solid phase extraction was used to remove very polar compounds and apolar pigments (21). Enriched extracts were diluted in final volumes proportional to their initial masses, allowing the injection of equivalent amounts of extracts in constant injection volumes. UPLC-TOF-MS analyses were performed on a Micromass quadrupole TOF Premier mass spectrometer from Waters. A collision energy ramp was applied from 15 to 35 eV. Argon was used as collision gas at a flow rate of 0.3 ml/min.

**Semi-preparative Liquid Chromatography MS Jasmonate Isolation**—Monitoring was performed on a Finnigan MAT (San Jose, CA) triple quadrupole mass spectrometer (TSQ700) equipped with a Finnigan electrospray ionization interface and coupled to a Varian modular HPLC system (Palo Alto, CA) with a Varian 9012 pump. Electrospray ionization conditions were capillary temperature 200 °C, source voltage 4.5 kV, acquisition in negative ion mode, full scan m/z 100–1000 Da, scan time 1 s. The isolation was achieved in isocratic mode using two Waters Xbridge BEH C₁₈ columns (10 × 250 mm, 5 μm) coupled in series. The isocratic conditions were identical to those used in UPLC-TOF-MS except for the flow rate (2.0 ml/min). An adjustable flow splitter Quicksplit from ASI (El Sobrante, CA) was used to split 1:50 of the flow to the MS detector.

**CapNMR Measurements**—CapNMR analyses were performed on a Finnigan Unity Inova 500 MHz NMR instrument (Palo Alto, CA) equipped with a 5-μl microflow CapNMR probe from Protasys/HRM Corp. (Savoy, IL) having an active volume of 2.5 μl. The samples were dissolved in 6.5 μl of CD₃OD and parked in the probe with a push volume of 9 μl. The signal of CD₃OD (¹H 3.31 ppm, ¹³C 49.00 ppm) (23) was used as reference (temperature 30 °C).

**JA Quantification**—The kinetics of JA accumulation in the wounded leaves of wild type (WT) and coi1–1 (19) were first established with the Weber et al. (10) protocol combined with an oxygen-18 JA internal standard (22). This procedure was then compared with the ultrarapid harvesting, extraction, and analysis described by Glauser et al. (21) and herein.

**RESULTS**

**Profiling of Oxygenated Fatty Acids by UPLC-TOF-MS Metabolomics**—A non-targeted metabolomic approach using UPLC-TOF-MS was devised to search for wound-inducible metabolites in *A. thaliana* (24). The profiles of wounded and unwounded WT leaves (Col-0 accession) as well as those of the JA synthesis mutant opr3 (20) in the Wassilewskija background were compared for the detection of jasmonates. Time series experiments (up to 24 h after wounding) were carried out to study the dynamics of jasmonate accumulation in wounded leaves and midveins. Additional time points (up to 45 min) were investigated in wounded leaves to evaluate shorter term metabolite accumulation related to wounding. Because a large number of analyses were conducted, a rapid UPLC method was
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devised. The main criteria for the search for jasmonates were strong induction in wounded Col-0 plants relative to control Col-0 samples and absence in control and wounded mutant opr3. A differential data treatment of the UPLC-TOF-MS analyses applied to all samples revealed the presence of known jasmonates in wounded Col-0 plants, such as JA (retention time (Rt) 2.41 min; m/z 209.1176; error 1.0 ppm), JA-Ile (Rt 3.04 min; m/z 322.2026; error 2.5 ppm), and JA-glucose (Rt 1.95 min; m/z 371.1695; error 3.0 ppm), as well as unidentified induced compounds of higher polarity. In particular, deprotonated molecules [M-H]− of m/z 225.1121 (Rt 1.37 min), m/z 338.1974 (Rt 2.05 min), and m/z 352.1757 (Rt 2.05 min) were detected. Molecular formulae could be deduced for these compounds with a good degree of confidence. C15H17O4 was associated to m/z 225.1121 (error 2.7 ppm), indicative of a hydroxylated jasmonic acid (HOJA). The ion m/z 338.1974 was defined as C15H22NO6 (error 2.1 ppm) and m/z 352.1757 as C15H22NO6 (error 0.9 ppm). Complementary MS/MS experiments using quadrupole-time-of-flight mass spectrometry revealed a leucine or isoleucine moiety (m/z 130.0869; error 0.8 ppm) for m/z 338.1974 and m/z 352.1757. Fig. 1, A and B, shows the MS/MS product ion spectra of both ions. Compared with JA-Ile fragmentation, the neutral loss for the transition m/z 338.1974, >m/z 130.0869 (208.1106 Da), differed by one oxygen atom (15.9949 Da) and could be related to HOJA. In the case of m/z 352.1757, the difference of 29.9742 Da (+2 oxygens and −2 hydrogens in molecular formula) suggested further oxidation of the JA moiety. However, the complete characterization of both compounds had to rely on CapNMR (25), and thus their isolation from larger quantities of wounded plant material (500 g) was needed.

Isolation and Identification of Oxygenated Jasmonates—Chromatographic conditions for isolation were optimized using UPLC-TOF-MS and then transferred geometrically to semi-preparative scale according to previously reported procedure (21). For m/z 225.1121, four peaks were detected and isolated (1, 2, 3, and 4, Rt 170.6, 176.9, 182.1 and 187.9 min, respectively), corresponding to four putative isomers of hydroxyljasmonamic acid. Two isomers were separated for both m/z 338.1974 (5 and 6, Rt 325.9 and 339.3 min, respectively), and m/z 352.1757 (7 and 8, Rt 332.0 and 358.6 min, respectively).

Based on CapNMR spectra, 1 was assigned to 11-hydroxyjasmonamic acid with a cis (Z) configuration of the cyclopentanone ring (cis-11-HOJA). Compound 2 was characterized as the corresponding trans (E) isomer (trans-11-HOJA). Absolute configuration assignment was not possible by NMR. The cis form was assumed to be (+)-(3R,7S) and the trans form (−)-(3R,7R) in accordance with known configurations for JA (13). The same statement is valid for all other compounds described in this report. A modification of the hydroxyl position was observed for compound 3, which was defined as cis-12-HOJA. Finally, compound 4 was the trans-12-HOJA. The complete 1H and 13C signal assignments for compounds 1–4 are summarized in Table 1. cis-12-HOJA (3) appeared to be much more abundant than trans-12-HOJA (4). Purified compound 3 was slowly converted into 4 after a few days in MeOH solution, which confirmed the cis (unstable) and trans (stable) configuration of 3 and 4, respectively. We observed small amounts of 11-HOJA in commercial JA samples. To ensure that 11-HOJA formation was not artifactual in our experiments, JA solutions were purified by HPLC and subjected to the sample preparation and analytical procedure. No trace of 11- or 12-HOJA was detected. Spiking experiments involving the addition of JA to control plants just before extraction did not lead to detectable artifact formation.

Two MS/MS product ion spectra of deprotonated ions [M-H]− of m/z 338.1974 and 352.1757 are shown in Fig. 1A and B, respectively. The main fragment of m/z 338.1974 is 322.2026 (15.9949 Da) and could be related to HOJA. In the case of m/z 352.1757, the difference of 29.9742 Da (+2 oxygens and −2 hydrogens in molecular formula) suggested further oxidation of the JA moiety. However, the complete characterization of both compounds had to rely on CapNMR (25), and thus their isolation from larger quantities of wounded plant material (500 g) was needed.

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FIGURE 1. Identification and structural analysis of hydroxylated jasmonates and a novel dicarboxyjasmonate. A and B, quadrupole TOF-MS/MS product ion spectra of deprotonated ions [M-H]− of m/z 338.1974 and 352.1757, respectively. The main fragment (m/z 130.0869) corresponds to a leucine or isoleucine moiety in both cases, based on exact mass measurements. Isoleucine was confirmed by further capillary NMR experiments. C, structures of all identified jasmonates. *, configuration of the cis (Z) form is assumed to be (+)-(3R,7S) and that of the trans (E) form (−)-(3R,7R) as in the case of JA (13). **, stereochemistry at C-11 has not been determined.
NMR Characterization of Jasmone Conjugated with Isoleucine and a Novel Jasmonate—CapNMR experiments enabled the assignment of the substructure corresponding to 12-HOJA for both 5 and 6. As for 3 and 4, the difference between both isomers was due to an epimerization of the cyclopentanone ring from a cis (6) to trans (5) configuration. The conjugated amino acid was identified as isoleucine in HSQC and COSY experiments. Therefore, 5 and 6 were determined as trans and cis 12-hydroxyjasmonic acid conjugated with isoleucine (12-HOJA-Ile). Table 1 presents all 1H and 13C signal assignments for both epimers.

CapNMR spectra of 7 and 8 were very similar to those of 5 and 6. The only significant difference was a shift of the methylene H-11 (δ 3.09) compared with that of 5 and 6 (δ 2.32) and the absence of signal for proton for proton C-12. The olefinic proton H-10 was also slightly shifted down field (δ 5.67). A COSY experiment showed a correlation between the methylene H-11 (δ 3.09) and the olefinic H-10 (δ 5.43). The molecular formula (C16H28NO5) deduced from TOF-MS measurement indicated that compared with 12-HOJA-Ile (C14H23NO3), an additional oxygen was present and two hydrogens were missing. From CapNMR, this difference in MS could be only explained by a carboxylic group at C-12.

Spatial Distribution—Analysis of the distribution of JA and its metabolites showed that the jasmonates fell into two categories, those which accumulated in the whole wounded leaf and midveins from the wounded leaf (HOJAs, 12-HOJA-Ile) and those largely absent from the midveins of the wounded leaf but present in the whole wounded leaf (JA, JA-Ile, 12-HOJA-Ile). The ability of HOJAs and 12-HOOCJA-Ile to accumulate in midveins did not correlate with their retention times in reverse-phase chromatography at pH 2.7 (Fig. 3A). However, during chromatography at physiological pH, the two compounds eluted prior to other jasmonates studied (Fig. 3B). During these studies with the ultrarapid sample preparation method we were unable to detect JA in resting leaves. However, basal levels of JA are often reported (e.g. 10). Comparison of the ultrarapid harvesting and extraction method with the previously published extraction and analysis method of Weber et al. (10) was undertaken. First, JA accumulation in unwounded and wounded WT and coi1–1 leaves was quantitated by gas chromatography MS (Fig. 4A). JA levels in both genotypes were similar, although slightly lower in coi1–1 leaves. JA in wounded leaves was quantitated by gas chromatography MS (Fig. 4B).
leaves and weighing them to the freezing step. When leaves were cut and frozen 1.2 min later and then analyzed by UPLC-TOF-MS, the JA level was seven times the signal-to-noise ratio. With the ultrarapid protocol used herein the time taken to cut and freeze leaves was an average 3 s and no JA was detectable in the samples (Fig. 4B).

These results raised the possibility that low levels of JA within the limit of detection of the Glauser et al. (21) method might be detectable within minutes not only in wounded leaves but also in leaves distal to the wound. Plants were wounded on the leaf apex (5 leaves/plant) and harvested after 2 or 5 min. Extracted ion chromatograms at m/z 209.118 ± 0.03 Da from extracts of unwounded plants, wounded leaves, and leaves distal to the wound (Fig. 5A) revealed a signal for JA in leaves distal to the wound. A replicated series of samples was used to generate semi-quantitative data based on signal-to-noise ratios. Levels of JA were undetectable in resting leaves but equal to 48 and 15 times signal-to-noise ratios, respectively, in wounded and distal leaves at 5 min after a wound (Fig. 5B).

**DISCUSSION**

State-of-the-art UPLC-TOF-MS and a very rapid sample preparation procedure were combined to study the production of jasmonate metabolites in wounded plant tissues. This non-targeted approach (24) revealed well known jasmonate metabolites and also led to the discovery of a hitherto unknown molecule. The known jasmonate derivatives identified were JA itself, jasmonoyl-isoleucine (JA-Ile) and 12-hydroxyjasmonoyl-isoleucine (12-HOJA-Ile), 12-hydroxyjasmonate (12-HOJA), and 11-hydroxyjasmonate (11-HOJA). JA-Ile is a biologically active form of jasmonate (12). 12-HOJA-Ile has recently been reported to be a wound-induced substance in tomato leaves by comparison with a synthetic standard (15). NMR data for the native compound isolated from plant tissues were lacking, and we were able to conduct a thorough CapNMR analysis confirming both the amino acid identity and the presence of cis and trans isomers. 12-HOJA has been studied extensively in *Arabidopsis* (16), and JA hydroxylation is thought to function as a means of inactivating JA. Interestingly, 12-HOJA was also shown to activate a hydroxyjasmonate sulfotransferase gene, showing that this compound has a potentially specific action in gene expression (16). As mentioned above, cis-12-HOJA (3) appeared to be much more abundant than trans-12-HOJA (4) during our analyses. This indicated a minor epimerization from cis to trans forms. These results suggest that hydroxylation of JA occurs before epimerization. 11-HOJA has already been reported to be a native substance in the leaflets of *Solanum demissum* (26). The hydroxylation of JA supplied to a strain of *Aspergillus niger*, which is not able to form jasmonate itself, yielded 11-HOJA (27). We are unaware of reports of 11-HOJA in *Arabidopsis*, but the compound was readily detectable after wounding in our experiments.

The discovery of 12-HOOCJA-Ile is of interest. The molecule is the first dicarboxylic jasmonate derivative known and thus has a high polarity. This compound was discovered in the wounded leaves relatively late with a maximal abundance at 6 h after wounding. This raised the questions of where, when, and how jasmonate metabolites including 12-HOOCJA-Ile accumulate in wounded plants.

**Accumulation of Jasmonate Derivatives in Space**—Four tissues were examined after wounding in extended time series...
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FIGURE 3. The polarity of jasmonates at physiological pH correlates with their appearance in midveins. A, elution of different jasmonates at pH 2.7. B, elution of different jasmonates at pH 7.2. Extracted ion chromatograms are displayed. Although 12-HOJA-Ile and 12-HOOJA-Ile coelute in A, a significant difference in retention is observed in B. The approximate polarity threshold indicated correlates with the accumulation of HOJAs and 12-HOOJA-Ile in midveins. Other jasmonates below the polarity threshold are not enriched in midveins.

these compounds in the wounded leaf was estimated at $\sim 1/10$, based on relative peak area comparisons. In contrast, HOJAs and 12-HOOJA-Ile were as abundant in whole wounded leaves as they were in the midveins from wounded leaves (ratio $\sim 1/1$). Also, they were clearly present in the midveins from leaves distal to the wound. Therefore, the molecules investigated fell into two clear categories according to their presence or apparent absence in the midveins of wounded leaves. What controls the spatially unequal distribution of jasmonate metabolites?

Factors Governing the Distribution of JA Metabolites—The most obvious differences between the jasmonates studied were in their physicochemical properties. We tested how these properties affect the distribution of this class of oxylipins. At pH 2.7,
all jasmonates are completely protonated, explaining the similar polarity of 12-HOOCJA-Ile and 12-HOJA-Ile in reverse-phase UPLC (i.e. similar retention times; Fig. 3A). However, the pH in cellular compartments where JA derivatives are enzymatically generated and metabolized is likely to be closer to neutrality. Assuming that the average pKₐ is ~4.5 for a carboxylic acid, non-esterified jasmonate derivatives should be thus completely deprotonated in most intracellular environments under physiological conditions. At near neutral pH, 12-HOOCJA-Ile behaved as a highly polar molecule and eluted much more rapidly than 12-HOJA-Ile (Fig. 3B).

The addition of a single hydroxyl group to JA greatly increased its polarity (Fig. 3B). This correlated with a radical difference in the distribution of JA and HOJA, with the latter compound found in relatively high abundance in the midveins of wounded plants (Fig. 2C). Concerning JA-Ile, hydroxylation of the jasmonoyl moiety at C-12 increased the polarity of JA-Ile. Because JA-Ile is even less polar than JA, this relative change in polarity was probably not sufficient for the accumulation of 12-HOJA-Ile in the midveins. However, further oxidation of 12-HOJA-Ile leading to 12-HOOCJA-Ile production would allow the accumulation of this molecule in midveins. The detection of the latter compound in midveins (Fig. 2E) and its delayed accumulation compared with 12-HOJA-Ile (Fig. 2G) are consistent with this hypothesis. Also in accordance with these observations is the fact that trihydroxy fatty acids have been observed to accumulate in broad bean (*Vicia faba*) leaves distal to infection sites (28). To assess whether midvein tissue was representative of the situation in the entire unwounded part of the wounded leaf, a further set of analyses was conducted. These analyses gave essentially the same results as those of midveins presented in Fig. 2. We also examined roots (0, 3, and 6 h after wounding leaves) for the presence of jasmonate metabolites. These analyses showed that the polar jasmonates 12-HOJA and 12-HOOCJA-Ile were present at low levels in roots (at ~1/10 of the levels of the compounds in wounded leaves, i.e. similar to the level observed for distal midveins). In conclusion, after wounding, the more polar jasmonate metabolites accumulate in the midveins of both wounded leaves and distal leaves.

**Rapid Systemic JA Accumulation**—Time course data revealed interesting temporal relationships between the different jasmonates. JA accumulated to peak levels at ~90 min following the wound (Fig. 2A). A closely similar time course of JA accumulation was reported previously (11). Of interest is the fact that JA accumulated rapidly in the wounded leaf and was readily detectable in the first 5 min after wounding (Fig. 2F). In agreement with quantitative data in Fig. 4A, 12-HOJA, which is thought to be a metabolite of JA (29), appeared only ~45 min after wounding. Another compound showing a fast accumulation (~5 min) in the wounded leaf was JA-Ile (Fig. 2G). The times necessary for the accumulation of 12-HOJA-Ile (~45 min) and 12-HOOCJA-Ile (~45 min) suggest that they are likely to be metabolites of JA-Ile. It is possible that 12-HOOCJA-Ile might be formed from further oxidation of the hydroxyl group of 12-HOJA-Ile. Levels of the compounds were maximal ~3 h after wounding for JA-Ile and 12-HOJA-Ile and ~6 h for 12-HOOCJA-Ile. These data thus help establish the temporal dynamics of jasmonate metabolite production in the wounded leaf. In summary, the polar metabolites start to accumulate at ~45 min post wounding. This marks entry into the enzyme-mediated jasmonate clearance phase. As the increase in JA and JA-Ile levels was even more rapid, further experiments were conducted in order to better characterize the timing of JA accumulation in response to wounding.

JA accumulation in leaves was first quantitated with a standard protocol (10) described in Mueller et al. (22). Consistent with earlier publications (10), a significant level of JA was measured in resting WT leaves (0.61 nmol/g fresh weight). However, we noticed that JA was not detectable in resting WT leaves with the ultrarapid sample preparation method. This was an interesting observation given that the two methods (10, 21) have similar detection limits for JA (see “Experimental Procedures”). This led to the hypothesis that some JA may accumulate in the time taken to weigh leaf tissue with the Weber et al. (10) protocol. Tests showed that this was indeed the case (Fig. 4B). The rapid Glauser et al. (21) protocol allowed us to explore JA accumulation in the leaves distal to the wound site. The tips of five
leaves (about half of the largest leaves in the rosette) of individual 5-week-old plants (average diameter 8 cm) were wounded. These leaves, as well as the leaves distal to the wound, were harvested 5 and 2 min after wounding and extracted using the ultrarapid procedure. JA was found to accumulate in the wounded leaf and in leaves distal to the wound where JA levels were 15-fold the signal-to-noise (s/n) ratio 5 min after wounding. In coil–1, JA levels distal to the wound reached an s/n ratio = 6 at 5 min after wounding; stimulus transfer appears to be largely COI1-independent. Estimation of the speed of JA accumulation in leaves distal to the wound site depends on where the s/n threshold for JA detection is set, and s/n is somewhat variable between experiments. Remarkably, JA accumulated to 4-fold s/n ratio in the undamaged leaves of WT plants only 2 min after wounding. Although we cannot exclude the possibility that some JA accumulation following wounding is due to induced inhibition ofJA turnover, JA synthesis (or release) likely contributes to the JA we detect.

There is evidence consistent with the long distance wound-initiated transport of jasmonates in tomato (8) and that exogenous methyl jasmonate is mobile in the vasculature of tobacco (9). Our results in Arabidopsis cannot distinguish whether JA (or a JA derivative) is transported from the wounded leaf to distal leaves or whether another signal such as a turgor pressure change or an ion flux (or a combination of these) is responsible for rapid (<2 min) distal JA accumulation. The phloem of wheat can transport small molecules at up to 0.7 cm/min (30). In our experiments, the average distance from the wounded area of the Arabidopsis leaves to the middle of the lamina of the unwounded leaves was ~6 cm, meaning that a stimulus travels at a minimum of 3 cm/min to result in JA accumulation in leaves distal to the wound. The possibility that this fast stimulus is not a mobile jasmonate in Arabidopsis must be considered.

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