Plant defense in the absence of jasmonic acid: The role of cyclopentenones

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The *Arabidopsis opr3* mutant is defective in the isoform of 12-oxo-phytodienoate (OPDA) reductase required for jasmonic acid (JA) biosynthesis. Oxylipin signatures of wounded *opr3* leaves revealed the absence of detectable 3R,7S-JA as well as altered levels of its cyclopentenone precursors OPDA and dinor OPDA. In contrast to JA-insensitive *coi1* plants and to the *fad3 fad7 fad8* mutant lacking the fatty acid precursors of JA synthesis, *opr3* plants exhibited strong resistance to the dipteran *Bradysia impatiens* and the fungus *Alternaria brassicicola*. Analysis of transcript profiles in *opr3* showed the wound induction of genes previously known to be JA-dependent, suggesting that cyclopentenones could fulfill some JA roles *in vivo*. Treating *opr3* plants with exogenous OPDA powerfully up-regulated several genes and disclosed two distinct downstream signal pathways, one through COI1, the other via an electrophile effect of the cyclopentenones. We conclude that the jasmonate family cyclopentenone OPDA (most likely together with dinor OPDA) regulates gene expression in concert with JA to fine-tune the expression of defense genes. More generally, resistance to insect and fungal attack can be observed in the absence of JA.

OPDA reductase | *opr3* | *Arabidopsis* | insect

A major objective in plant biology is to develop an integrated understanding of how plants survive in their environment and reproduce. Although it has become clear in the last decade that jasmonic acid (JA) is a key regulator in the development, physiology, and defense of plants, the complexity of the signaling network in which JA evolves is just emerging (1). JA is involved in carbon partitioning (2), in mechanotransduction (3), and the ability of plants to synthesize and perceive JA is absolutely essential for the correct development and release of pollen in *Arabidopsis* (4–7). Highlighting the regulatory importance of JA, a JA-responsive transcription factor, ORCA3, first found in *Catharanthus*, provides an important link between primary and secondary metabolism (8). There is also strong evidence supporting a central role of JA in plant defense. Exogenous JA powerfully regulates the expression of many defense genes in plants, and its *in vivo* production and perception seem to be of vital importance in mounting successful defense against insect attackers (9–11). Together with ethylene, JA also plays a crucial role in defense against necrotrophic fungi (12–14) and in induced systemic resistance in response to nonpathogenic rhizobacteria (15). Broader roles of JA in plant stress responses are likely; it is known that the JA biosynthesis pathway is important in gene activation subsequent to UV damage in plants (16), and JA has been implicated in some responses to water stress (17).

The biosynthesis of JA occurs through the octadecanoid pathway (18, 19) and is initiated by the addition of molecular oxygen to linolenic acid (18:3) to form 13-hydroperoxylinolenic acid (13-HPOTrE). This fatty acid hydroperoxide is then dehydrated by allene oxide synthase (AOS) and cyclized by allene oxide cyclase (AOC) to the cyclopentenone 12-oxo-phytodienoic acid (OPDA). Although the chemical nature of OPDA allows four stereoenantiomers, the concerted action of AOS and AOC generate exclusively 9S,13S-OPDA (20), the precursor of active 3R,7S-JA (21). The next step in the formation of JA is the reduction of the pentacyclic ring double bond in 9S,13S-OPDA by the enzyme OPDA reductase 3 (OPR3; ref. 22) to the cyclopentanone 3-oxo-2(2′[*Z*]-pentenyl)-cyclopentane-1-octanoic acid (OPC:8). Other related enzymes such as OPR1 and OPR2, initially thought to be involved in the biosynthesis of active 3R,7S-JA, have almost no activity against 9S,13S-OPDA (22). Finally, OPC:8 is shortened by three cycles of β-oxidation to yield JA. In parallel, hexadecatrienoic acid (16:3) can be metabolized to dinor oxo-phytodienoic acid (dnOPDA; ref. 23), the 16-carbon structural homolog of OPDA.

Although attention has focused mainly on JA as a signal, the possibility that the JA precursor OPDA could itself be biologically active has been proposed (3, 23–25). However, there is also strong evidence, based on detailed pharmacological studies, that the natural oxylipin capable of eliciting the synthesis of alkaloids like sanguinarine in *Eschscholtzia* cell cultures is JA (26). Until recently, no tools have been available to perform genetic tests with which to resolve which jasmonate family member(s) is active *in vivo*. The availability of *Arabidopsis* plants lacking a functional *OPR3* gene (*opr3*, ref. 7; *dde1*, ref. 6) has now permitted the genetic separation of OPDA and JA effects *in vivo*. In the *opr3* mutant JA cannot be produced; these plants are male sterile because they produce predominantly inviable pollen and have delayed anther dehiscence. Male sterility in these plants is rescued efficiently by JA but not by OPDA, ruling out an active role for OPDA in pollen development (6, 7). Thus evidence based on well characterized mutants indicates an exclusive role of JA in male gametophyte development in *Arabidopsis*.

This study addressed the question: Is JA the sole octadecanoid-derived effector necessary for defense in plants? By using the *opr3* mutant we were able to investigate the effects of OPDA on gene expression without having to consider OPDA conversion to JA. The results demonstrate that OPDA is itself a potent gene regulator in the wound response and that protection of plants against the attack of an insect or a fungal pathogen is obtained in the absence of JA.

Materials and Methods

Plants, Insects, and Pathogens. *Arabidopsis thaliana* were grown under a 9-h [120 μE (1 mol of photons) m^{-2} s^{-1}]/15-h light/dark photoperiod. Wild-type (ecotype Wassilewskija, WS), *opr3* (in the WS background), *coi1–1*, and *fad3–2 fad7–2 fad8* mutant plants were grown in pasteurized soil (12). Mutants *coi1* and *fad3 fad7 fad8* are in a Columbia background (Col-0); this ecotype is known to be resistant to attack by *Bradysia*

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Abbreviations: JA, jasmonic acid; OPDA, 12-oxo-phytodienoic acid; OPR, OPDA reductase; dnOPDA, dinor OPDA.

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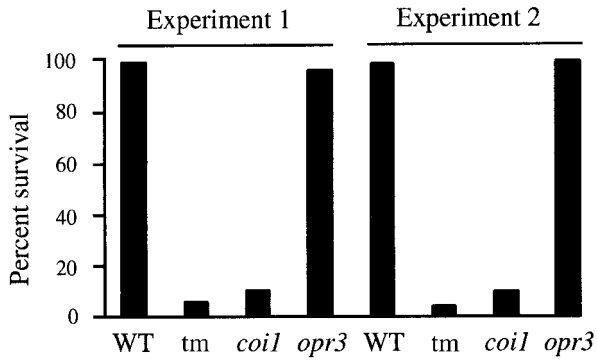

Fig. 1. *opr3* mutant plants are not susceptible to attack by *Bradysia*. Results of two independent experiments in which a mixed population of approximately 150 wild-type (WT), *fad3 fad7 fad8* triple mutant (tm), *coi1*, and *opr3* plants were grown in a net enclosure and subjected to heavy infestation with *B. impatiens* larvae. As the seedlings reached the four-leaf stage, adult *Bradysia* flies (average 50–80) were introduced in the net every other day over a 2-week period. Damage to the petiole or to the roots of the plants by *Bradysia* larvae resulted in wilting followed by death of the plants. After bolting, the percentage of surviving plants for each genotype was calculated.

impatiens larvae (10). *B. impatiens* (Diptera; Sciaridae) were maintained on wild-type *Arabidopsis* and, for experiments, adult flies were harvested and used to challenge plants as described in Fig. 1. *Alternaria brassicicola* (MUCL 20297) was from the Mycothèque Université Catholique de Louvain (Belgium) and was maintained on potato dextrose agar at 26°C. Conidia/conidiospores were collected in distilled water 10 days after subculture, and infection was performed by applying droplets (5 μl) of an aqueous solution containing 5×10^5 spores·ml^{-1} of *A. brassicicola* onto leaves of four- to six-leaf-stage plants.

cDNA Microarray Analysis and RNA Gel-Blot Analyses. Established cDNA microarray protocols were used (27). Full details of the cDNA microarray used as well as expressed sequence tags (EST) accession nos. are given in Reymond *et al.* (28), at www.unil.ch/ibpv, and in Table 1, which is published as supporting information on the PNAS web site, www.pnas.org). Based on previous results (28), the following genes were used as negative controls in wounding experiments: plastocyanin, *α-TUB*, *β-TUB*, *EF1*, and *ACT*. Wound-inducible positive control genes were *JR3* and *TSA* (COI1-dependent) and *GST1*, *XERO2*, and *RNS1* (COI1-independent; ref. 28). A 2-fold change in transcript level was regarded as significant (28); however, for key interpretations, genes for which larger changes in transcript level occurred were selected. In brief, mRNA samples (2 μg) from wild-type or *opr3 Arabidopsis* leaves were converted into fluorescently labeled single-stranded cDNAs by direct incorporation of either Cy3-dCTP (control) or Cy5-dCTP (wounded or treated with either JA or OPDA). These cDNA populations were hybridized simultaneously to cDNA microarrays, and the fluorescent signal for both fluors was recorded and quantitated. Two independent experiments for each wounding time point and three for treatment of *opr3* with OPDA or JA were carried out. Cluster analyses (29) were used to display data from time courses or to compare individual experiments. RNA gel-blot analyses were conducted as described (30). For *PDF1.2* gel-blot analyses, EST accession no. T04323 was used. The loading control probe was for the *Lhb1B2* gene (EST accession no. R89981), encoding chlorophyll a/b-binding protein. Both probes were labeled with digoxigenin (Roche Molecular Biochemicals) by PCR amplification.

Oxylipin Signatures and Treatments. Oxylipins from *Arabidopsis* leaves (1 g) were extracted according to Weber *et al.* (23). Approximately 350 nmol·g^{-1} tissue of OPDA (Cayman Chemicals, Ann Arbor, MI) and JA (Sigma) were sprayed onto plants in an acetone carrier (30), and leaves were harvested after 4.5 h. Control plants were sprayed only with carrier.

Results

***opr3* Plants Resist Attack by an Insect and a Fungal Pathogen.** The previous characterization of the *Arabidopsis opr3* mutant indicated that JA is the signaling molecule required to induce pollen maturation and release; OPDA could not substitute for JA in this role (7). Several lines of evidence suggest that JA is also a physiological signal for activation of genes involved in the defense of plants against insect attack (9, 10) and infection by some fungal pathogens (12–14). In particular, a triple mutant that lacks the fatty acid precursors of JA synthesis, *fad3 fad7 fad8*, and the JA-insensitive mutant, *coi1*, have both been shown to be extremely susceptible to insect attack (ref. 10 and this study). Moreover, almost wild-type level of protection is provided to the *fad3 fad7 fad8* mutant by exogenous JA (10, 12). On the basis of these observations, it was expected that the *opr3* mutant would also be defective in defense responses. However, casual observation suggested that *opr3* plants were not susceptible to insect and pathogen damage. To further investigate this observation, we conducted two independent experiments in which wild-type, *fad3 fad7 fad8*, *coi1*, and *opr3* plants were grown in mixed stands and challenged with a heavy population of *B. impatiens*, the common fungal gnat, as they reached four-six leaves (Fig. 1). By the time the plants were flowering, only 4–6% of *fad3 fad7 fad8* and 10–11% of *coi1* plants had survived. Death occurred as a result of mechanical damage caused by *Bradysia* larvae chewing on the lower leaves, stems, petioles, and roots of the plants. In contrast, both wild-type and *opr3* plants remained largely undamaged, with only one fatality in 146 and 158 plants, respectively. Because *opr3* plants looked as healthy as the wild type, and because death rates in *fad3 fad7 fad8* and in *coi1* were substantial, it can be concluded that *opr3* is resistant to *Bradysia*. To investigate the effects of the *opr3* mutation on defense responses of *Arabidopsis* against a necrotrophic fungi, plants of the same four lines were infected with the fungal pathogen *A. brassicicola* (14). *A. brassicicola* caused leaf damage and necrosis of the inoculated leaves in all four lines. However, 3 weeks after infection, both wild-type and *opr3* plants remained viable with more than 60% of their leaves being green and showing few or no symptoms. By contrast, over 90% of leaves on *fad3 fad7 fad8* and on *coi1* plants were dead or severely chlorotic (data not shown). These results show that *opr3* plants retain resistance to fungal infection as well as to insect attack.

***opr3* Mutant Plants Do Not Synthesize 3R,7S-JA.** Previous results indicated that, in the *opr3/dde1* mutant, *OPR3* transcript is absent in both reproductive and vegetative tissues (6, 7). The mutant also lacks detectable OPDA reductase activity against 9S,13S-OPDA (6), the main OPDA enantiomer in plants and the precursor of active 3R,7S-JA (21), and all available evidence points to an essential role for OPR3 in OPDA reduction (6, 7, 22). However, because *opr3* resists attack, a possibility remains that an as yet uncharacterized OPR isoform is capable of replacing *OPR3* in vegetative tissues and producing low levels of 3R,7S-JA. To test the possibility that *opr3* vegetative tissues convert OPDA into 3R,7S-JA, the levels of three JA family members (OPDA, dnOPDA, and JA) were measured in nonwounded and wounded leaves. As shown in Fig. 2*A*, nonwounded leaves of wild-type *Arabidopsis* contained less than 0.2 nmol/g of fresh weight of JA estimated from both *cis* 3R,7S and *trans* 3R,7R-JA enantiomers, and the level increased to 6 nmol/g of fresh weight 3 h after wounding, before declining to an intermediate value after 6 h. By contrast, in *opr3* JA levels were close to the detection limit of the mass spectrometric assay used, and

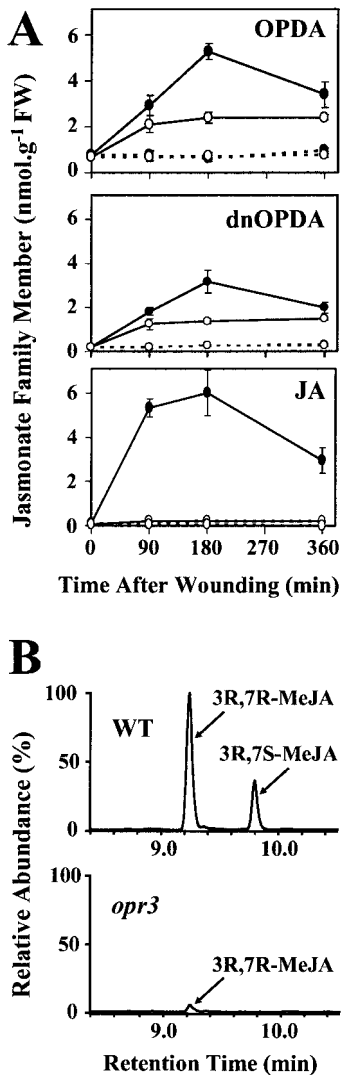

Fig. 2. Accumulation of jasmonate family members in wounded *Arabidopsis*. (*A*) Oxylipins were extracted from leaves at different times after mechanically wounding the leaf apex. Kinetics of OPDA, dnOPDA, and JA accumulation were followed in unwounded (dashed lines), wounded (solid lines), wild-type (solid symbols), and *opr3* (open symbols) plants. Data are the mean ±SE of three determinations. FW, fresh weight. (*B*) 3R,7S-JA is not detectable in the oxylipin signature of *opr3* leaves. Selective ion monitoring for the ion $m/z = 224$ revealed two methyl jasmonate (MeJA) isomers, 3R,7R-MeJA and 3R,7S-MeJA, in wild-type (WT) *Arabidopsis* leaf extracts 90 min after wounding but only trace levels of the 3R,7R enantiomer in *opr3* extracts.

increased from 0.03 nmol/g of fresh weight in nonwounded leaves to 0.2 nmol/g of fresh weight after wounding, which corresponds to less than 4% of the level present 3 h after wounding in wild type. Comparatively, the *fad3 fad7 fad8* mutant accumulated 0.07 nmol/g of fresh weight of JA and 0.11 nmol/g of fresh weight of OPDA 90 min after wounding (not shown). OPDA and dnOPDA accumulated in response to wounding in both wild-type and *opr3* leaves. The accumulation profiles were qualitatively similar in the two genotypes with the slight difference being that, in wounded *opr3* leaves, both cyclopentenones reached maximum levels between 90–360 min, whereas in wild-type leaves, the levels of the compounds had partially subsided 360 min after wounding. Clear quantitative differences in cyclopentenone levels were observed. In wounded wild-type leaves, OPDA levels were at their maximum at around 5 nmol/g of fresh weight at both the 90- and 180-min time points. In contrast, at the same time points, OPDA levels reached half of this value (≈2.5 nmol/g of fresh weight) in wounded *opr3* leaves. Similar behavior was noted for dnOPDA in wounded *opr3* leaves. To investigate further the isomeric composition of the trace level of JA in *opr3* leaves, selective ion monitoring of extracts produced 90 min after wounding (Fig. 2*B*) was effected. In addition to being at extremely low levels relative to wild type, JA in wounded *opr3* leaves occurred only in the *trans* (3R,7R) form, whereas both the *cis* (3R,7S) and *trans* (3R,7R) stereoenantiomers were present in the wild type (Fig. 2*B*) and in *fad3 fad7 fad8* (not shown).

Wound-Regulated Gene Expression in *opr3* Mutant Leaves. The *opr3* mutant is resistant to *Bradysia* attack and *Alternaria* infection, whereas both *fad3 fad7 fad8* and *coi1* plants are highly susceptible. The implication of these observations is that, whereas JA is not necessary to provide full resistance in *opr3*, another signal molecule(s) must function to activate defense responses in the mutant. To test this hypothesis, the impact of the *opr3* mutation on gene expression in wounded *Arabidopsis* leaves was assessed by using a dedicated cDNA microarray carrying about 150 defense-related genes. Samples were taken at 90, 180, and 360 min after wounding and, for each time point, at least two independent experiments were conducted. An average 2-fold increase in message level in at least 1 time point was taken as an indication of gene activation (28). Whereas the overall pattern of gene expression was very similar between wild-type and *opr3* plants after wounding, we observed that gene expression dynamics differed in the two genotypes (see http://www.unil.ch/ibpv and Table 2, which is published as supporting information on the PNAS web site). For example, 90 min after wounding 44 genes were up-regulated in wild-type plants, whereas in *opr3* plants, 38 genes were up-regulated. At 180 min after wounding, 42 and 18 genes were up-regulated in wild-type and *opr3* plants, respectively. By 360 min, 13 genes were up-regulated in wild-type vs. 3 in *opr3* plants. Fig. 3*A* shows a clustal analysis (29) of 24 representative genes in wounded wild-type and *opr3* leaves. Two groups can be distinguished. First, the top section consists of a group of *COI1*-dependent genes. As reported (28), all of these genes were activated after wounding in wild-type plants. For example, the changes varied from a weak increase in transcript level (2.2-fold for *ASB* after 180 min) to a strong increase (15.1-fold for *VSP* after 180 min). Interestingly, in *opr3* mutants, the clustal analysis revealed a gradient of induction ranging from genes for which no increase in transcript level was detected (*MBP*, *JIP*, *VSP*) through to genes that were equally activated in both *opr3* and wild-type plants, e.g., *COMT* (90 min after wounding there was an 8.2-fold increase in transcript in *opr3* and an 8.6-fold increase in transcript in wild-type leaves) and *CCR* (for which, 90 min after wounding, there was a 3.6-fold increase in transcript in *opr3* and a 3.7-fold increase in transcript in wild type). In between these extremes were genes like *JR3*. For this gene, 90 min after wounding, transcript increased 2.7-fold in *opr3* and 4.8-fold in wild-type leaves. Many other genes (e.g., *CYP83B1*, *HPL*, *TSA*) showed a significant but weaker activation in wounded *opr3* leaves. Second, a group of *COI1*-independent genes (Fig. 3*A Bottom*) had a highly similar expression behavior in both wild-type and *opr3* plants after wounding (e.g., *RNS1*, *OPR1*, *GST1*). Thus, these microarray results show that many genes known to be *COI1*-dependent were induced by wounding in a mutant lacking JA. As expected (28), several defense-related genes such as *PDF1.2* and *HEL* were not wound-inducible in wild-type or *opr3* genotypes (data not shown).

Because the *opr3* mutant cannot convert OPDA into JA, this plant offers the possibility to compare the effects of exogenous JA and OPDA on gene expression. The possibility that exoge-

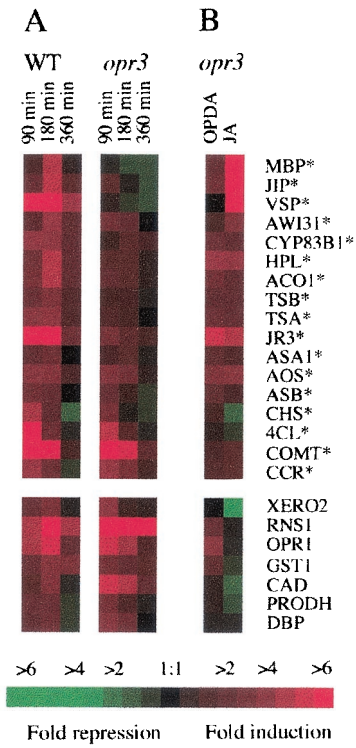

Fig. 3. DNA microarray analysis after mechanical wounding and application of oxylipins. Wound-inducible gene expression in wild-type (WT) and *opr3* *Arabidopsis* plants 90, 180, and 360 min after mechanical wounding (*A*) was compared with gene expression after treatment of *opr3* with OPDA or JA for 4.5 h. (*B*) A clustal analysis was performed (see *Materials and Methods*) on a set of genes of interest in this study. Each gene is represented by a single row of colored boxes, and each experiment is represented by a single column. Each experiment is the average of at least two replicates. All gene expression data can be found in Tables 1–3, which are published as supporting information on the PNAS web site, and at http://www.unil.ch/ibpv.

nous OPDA could induce *COI1*-dependent gene expression in *opr3* was then tested. For each treatment at least three independent experiments were conducted. As a positive control, we first verified that exogenous JA was able to induce many of the *COI1*-dependent genes in *opr3* (Fig. 3*B Top*). Indeed, genes such as *MBP*, *JIP*, *HPL*, *JR3*, or *VSP* showed an induction ranging from 3.5-fold (*HPL*) to 14.1-fold (*JIP*). When *opr3* plants were treated with OPDA, the same genes were induced, with the notable exception of *VSP*. Surprisingly, OPDA also induced three *COI1*-independent genes (*RNS1*, *OPR1*, and *GST1*), which in contrast were not up-regulated by JA (Fig. 3*B Bottom*). Furthermore, two more genes (*GST8* and *ELI3*) were induced only by OPDA and not JA, although they were not significantly induced by wounding (see http://www.unil.ch/ibpv and Table 3, which is published as supporting information on the PNAS web site).

Having shown that the induction of *COI1*-dependent genes still occurred in wounded *opr3* plants and that OPDA was potentially responsible for this induction, we examined whether JA production is necessary for the induction of defense genes after a fungal attack. Defense response against *A. brassicicola* is also mediated through the COI1 pathway, and expression of *PDF1.2*, which encodes a protein with demonstrated antifungal properties, is known to be powerfully up-regulated in a *COI1*-dependent manner in this pathosystem (14). *PDF1.2* transcript levels in wild-type and *opr3* plants infected with the fungus were monitored by using RNA gel-blot analysis. As shown in Fig. 4, the *PDF1.2* transcript is detectable in infected wild-type and *opr3*

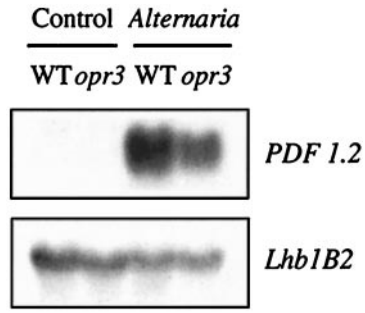

Fig. 4. *PDF1.2* transcript accumulation in wild-type (WT) and *opr3* plants challenged with *A. brassicicola*. RNA gel-blot analysis was performed by using 15 μg of total RNA isolated 48 h after infection. *PDF1.2* transcripts levels are shown in control tissue treated with water or inoculated with *Alternaria* as described (14). The *Lhb1B2* gene encoding a chlorophyll a/b-binding protein was used as a loading control.

leaves but not in control leaves from either genotype, demonstrating that in this system too, JA is not required for induction of *COI1*-dependent gene expression.

Discussion

Defense in the Absence of 3R,7S-JA. The disruption of the JA biosynthetic pathway leads to the extreme susceptibility of plants to attack by some insects (9, 10) and necrotrophic fungi (refs. 12 and 13 and this study). However, which molecule(s) in the pathway need to be synthesized to mount an active defense is unknown. The *opr3* mutant offers the unique possibility to test the effects of truncation of the biosynthetic pathway on plant survival from insect attack. Unlike the response of plants defective in the biosynthesis of all jasmonate precursors (*fad 3 fad 7 fad 8*) or of plants defective in JA perception (*col 1*), the *opr3* plants, which lack the ability to make wild-type levels of JA, survived as well as wild-type plants in the face of attack by caged *Bradysia* larvae (Fig. 1). Therefore, in the case of defense against this insect, JA production seems unnecessary. This implies that another member of the jasmonate family can replace JA in triggering defense in *opr3*. The replacement molecules must function by means of the COI1 protein, because the *coi1* mutant is susceptible to attack (Fig. 1). Another line of evidence comes from the observation that *opr3* and wild-type plants are more resistant to the fungal pathogen *A. brassicicola* than *coi1* and *fad3 fad7 fad8* mutants. More generally, the results demonstrate that protection against an insect or a fungal pathogen, and *COI1*-dependent defense gene expression, can be mediated by OPDA in the absence of JA. This conclusion is in contrast to the case of pollen maturation and anther dehiscence where JA production is an obligate requirement (6, 7).

Jasmonate Family Dynamics in *opr3*. Our analysis of the levels of two cyclopentenone jasmonate family members OPDA and dnOPDA, as well as the cyclopentanone JA (23), in wild-type and *opr3* plants in response to mechanical wounding (Fig. 2) showed that the pools of these compounds are dynamic, and in the wild type, responded to wounding by a 6-, 13-, and 30-fold increase of the levels of OPDA, dnOPDA, and JA, respectively, 3 h after wounding (Fig. 2*A*). In striking contrast, the *opr3* mutation prevented JA from accumulating after wounding, and the levels observed were slightly above detection limit of the method. The *opr3* mutation also resulted in a quantitative alteration in cyclopentenone accumulation. Levels of OPDA and dnOPDA in wounded *opr3* leaves reach about half their levels in wounded wild-type leaves. These results imply that cellular cyclopentenone levels are regulated, in part, by JA and/or other cyclopentanones [i.e., the oxo-pentenyl-cyclopentane (OPC) in-

termediates in JA synthesis]. In wild-type leaves, the amount of *cis* 3R,7S-JA vs. *trans* 3R,7R-JA occurred in a 1:3 up to a 1:2 ratio, whereas JA measured in *opr3* occurred exclusively in the *trans* 3R,7R configuration (Fig. 2*B*). Thermal conversion in the gas chromatograph injector port is responsible for much of the epimerization of 3R,7S-JA into 3R,7R-JA but in wild type and in *fad3 fad7 fad8* the former epimer is observed. The absence of detectable 3R,7S-JA in *opr3* leaves is highly significant because this isomer is believed to be biologically active unlike the 3R,7R enantiomer (21). Thus, like in floral organs, no biologically active JA is detectable in *opr3* leaves after wounding, ruling out the possible involvement of the additional OPR isoforms in active JA biosynthesis. Because both *OPR1* and *OPR2* were shown to be wound-inducible in *Arabidopsis* vegetative tissue (31), substrate specificity of OPR1 and OPR2 rather than enzyme availability is the limiting factor in the synthesis of active JA in *opr3* (although the possibility that OPR1 and -2 are not expressed in cell types capable of producing JA cannot be ruled out). These observations are strongly supported by the conclusion reached by Schaller *et al.* (22) that OPR3 rather than OPR1 or OPR2 is the form of OPR required for active JA biosynthesis. The putative fourth and fifth OPRs identified in the *Arabidopsis* database (GenBank accession nos. AAC33200 and AB010695) are more closely related to OPR1 and OPR2 (7, 19), and it can be assumed that they do not contribute to *in vivo* production of 3R,7S-JA. In summary, the leaves of *opr3* do not contain detectable 3R,7S-JA, making them ideal for the study of the role of JA in defense and gene expression.

OPDA as a Regulator of Gene Expression. The *opr3* mutant was used to investigate the role of JA and its precursors in signal transduction. With this mutant, the following questions were addressed: What role does JA play in the regulation of gene expression in vegetative tissues? Can OPDA (or one of its metabolites) contribute to gene regulation in the absence of JA? Three possibilities exist. Consistent with recent studies (6, 7, 26), JA in free or conjugated form could be uniquely capable of regulating gene expression. A second possibility is that OPDA (and not JA) is the sole *in vivo* regulator of gene expression and that effects of exogenous JA merely mimic the effects of OPDA or induce its synthesis. A third possibility is that cyclopentanones and cyclopentenones can both contribute to the regulation of gene expression. OPDA has been proposed to be the principal *in vivo* regulator of tendril coiling in *Bryonia dioica* (25, 32). Exogenous JA and OPDA induce different patterns of volatile production in lima beans (33). It has been proposed that JA and OPDA/dnOPDA (as well as related compounds) may all be *bona fide* regulators and that their changing proportions during disease or development would allow the cell to fine-tune gene expression (23, 34). Although this scenario, in which both JA and OPDA are biologically active, has received support (35), the current study allowed a direct genetic test of the hypothesis.

Gene expression profiles in wild-type and *opr3* leaves were measured after mechanical wounding (Fig. 3*A*). We found that transcript levels for many genes were elevated after wounding in both genotypes. This finding is remarkable, because several genes on the microarray that are up-regulated after the mechanical wounding of *Arabidopsis* leaves require a functional COI1 pathway (28). These genes should, in principle, require both functional JA biosynthesis and signal transduction pathways. However, a closer look at the *COI1*-dependent genes reveals that there is a gradient of wound induction in *opr3*. Some genes are powerfully up-regulated in wild-type plants but not in *opr3* (*MBP*, *JIP*, and *VSP*, for which there were, respectively, 4.3-, 4.7-, and 15.1-fold increases in transcript leaves 180 min after wounding). These are also strongly induced by JA treatment, suggesting that this group of genes is regulated to a large extent by JA *in vivo*. Several *COI1*-dependent genes were up-regulated in both

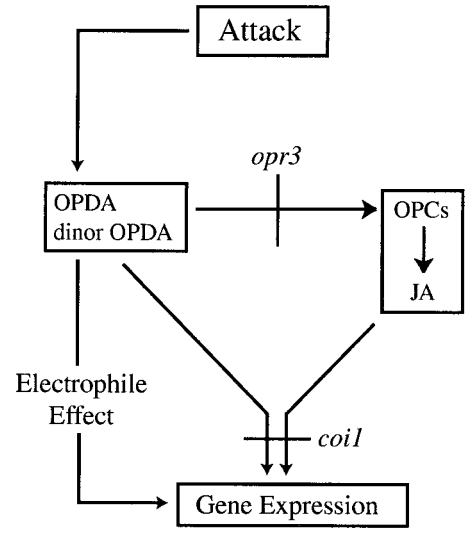

Fig. 5. Model for the role of jasmonate family members in the fine control of gene expression in wounded or diseased *Arabidopsis* leaves. The cyclopentenones OPDA and dnOPDA can regulate genes directly via a COI1-dependent pathway, or via the same signal pathway, after their conversion to cyclopentanones, i.e., JA or its oxo-pentenyl-cyclopentane (OPC) precursors. In this way, JA and OPDA could both regulate specific sets of genes or may act in concert to control the expression of common sets of genes. Another layer of regulation might be provided by the electrophilic properties of cyclopentenone jasmonates.

genetic backgrounds but to a lesser extent in *opr3* than in wild type (e.g., *JR3*, *HPL*, and *ACO1*). However, most of these genes were induced by treatment with JA or OPDA. This observation indicates that OPDA is a gene regulator *per se* during wounding and that both JA and OPDA are required for a full response. Decreased amounts of OPDA/dnOPDA in *opr3* after wounding as compared with wild type could account for reduced gene expression as could changes in the dynamics of the cyclopentenone pool in *opr3* plants. Finally, some *COI1*-dependent genes are similarly induced in wild type and *opr3* (e.g., *COMT*, *CCR*). Because these two members of the aromatic amino acid metabolism are not induced by application of JA or OPDA, it is possible that another signal molecule(s) controls the activation of these genes in a *COI1*-dependent manner. In summary, we show that OPDA is a potent gene regulator. We can exclude the possibility of the conversion of OPDA to JA in *opr3* plants, because *VSP*, which is strongly induced after wounding in wild type and by exogenous JA in *opr3*, was not induced in *opr3* by exogenous OPDA or after wounding (Fig. 3).

These observations were extended by investigating the expression of the *COI1*- dependent defensin gene *PDF1.2* during pathogenesis. *PDF1.2* transcript accumulated in both wild-type and *opr3* leaves infected with *A. brassicicola*, indicating that JA synthesis is not necessary for the activation of this gene. However *PDF1.2* induction was lower in *opr3* than in wild type (Fig. 4), suggesting again that JA in wild-type plants may coregulate or potentiate the activity of OPDA (and perhaps *vice versa*).

Electrophilic Properties of OPDA. Further supporting a role for OPDA as a gene regulator is the observation that it can activate genes such as *GST1*, *RNS1*, and *OPR1*. These genes are known to be up-regulated by wounding in a *COI1*-independent manner (28) but the signal molecule that controls their activation is not known. *GST1*, *RNS1*, and *OPR1* are induced by wounding in both wild type and *opr3* and they are not activated by JA (Fig. 3). It is possible that the expression of these genes depends, at least in part, on a pathway involving the electrophilic effect caused by the

chemistry of the cyclopentenone ring in OPDA. OPDA and dnOPDA are reactive electrophilic species possessing α,β-unsaturated carbonyl groups. Molecules containing this feature have been implicated as potent gene regulators in diseased plant tissues where they could also contribute to cell damage (36). Cyclopentenone prostaglandins also possess the α,β-unsaturated carbonyl feature and have been implicated as *in vivo* regulators (37). The results presented here indicate a new mechanism potentially responsible for the wound induction of several JA-independent genes. However, full demonstration of the relevance of the electrophilic properties of OPDA will require further investigation.

A model summarizing the roles of jasmonates in the control of gene expression in wounded or diseased *Arabidopsis* leaves is given in Fig. 5. We postulate that JA and OPDA/dnOPDA play distinct but complementary roles in the fine-tuning of gene expression as predicted in the oxylipin signature hypothesis (23). One pathway involving OPDA and JA clearly acts through the COI1 complex, but is it possible that at least one other pathway controlled by means of the electrophilic properties of cyclopentenone jasmonates might exist. This is the first report, to our knowledge, of such properties of cyclopentenones in plants. Interestingly, the chemical structure of the cyclopentanone ring prohibits this function for JA.

In conclusion, cyclopentenones and cyclopentanones can act alone or in concert to regulate gene expression. Both JA and OPDA are envisaged to have distinct and important roles in the control of gene expression in plant defense. The exact mechanism of this regulation is not known, but our results illustrate a complex network of signaling molecules in the jasmonate family. The successful genetic dissection of different regulatory activities for lipid-derived signals in plants should pave the way toward a better understanding of how plant cells generate and interpret complex information in the oxylipin signature.

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