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Involvement of Jasmonic Acid and Derivatives in Plant Responses to Pathogens and Insects and in Fruit Ripening

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Abstract

The jasmonate pathway plays a crucial role not only in defense against notorious pests and pathogens but also in plant development. In addition to the well documented evidence demonstrating the role of jasmonates in plant protection against bacteria and fungi, it has also become clear that induced resistance to herbivores in many, if not all, plants is mediated by the jasmonate pathway. This pathway plays important roles in defense against not only insects but also against at least one other class of arthropod, Arachnida. Jasmonates are produced naturally by climacteric and nonclimacteric fruits thereby inducing ethylene production and enhancing the production of the aromas in climacteric fruits. All these results together and advances in the manipulation of the pathway hold promise for future strategies in agriculture.

Key words: Oxylipins; Jasmonate; Pathogen response; Insects; Plant development; Fruit ripening

Introduction

Plant oxylipins constitute a group of bioactive fatty acid derivatives that perform several important roles in growth and development. A large body of research has focused on the jasmonate family of oxylipins, which includes jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA). These signaling compounds, collectively referred to as jasmonates (JAs), are cyclopentanone compounds and are ubiquitous in the plant kingdom. Because of their volatility, methyl jasmonates may be involved in interplant communication (Farmer and Ryan 1990). Some evidence has demonstrated its presence in fungi (Miersch and others 1999) and macroalgaes (H. Peña-Cortés unpublished results) as well. JAs are well characterized with respect to their role in regulating defense responses against herbivore attack and infection by some pathogens (Kessler and Baldwin 2002; Turner and others 2002; Wasternack and Hause 2002; Weber 2002). Moreover, accumulating evidence suggests that jasmonates have a role in plant resistance against abiotic stimuli such as mechanical stress (Weiler and others

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1993), salt stress (Dombrowski 2003), UV irradiation (Conconi and others 1996), low temperatures (Wasternack and Parthier 1997) and ozone exposure (Rao and others 2000).

Exogenous JAs lead to numerous induced and inhibitory effects on plant developmental processes (Creelman and Mullet 1997; Wasternack and Hause 2002; Farmer and others 2003). Correlations between endogenous JA levels in specific tissues and the effects of the applied hormone have provided evidence that JAs have a role in promoting senescence, fruit ripening, embryo development, and the accumulation of storage proteins (Creelman and Mullet 1997; He and others 2002). Most of those studies have been performed with a commercial, synthetic mixture that contains at least six different methyl jasmonates (Muller and Brodschelm 1994), but there is increasing evidence that the absolute configuration is of decisive importance for their physiological effects (Koda and others 1992). The same holds for their odor activity toward humans: (3*R*,7*S*)-Methyl jasmonate displays a strong and the 3*R*,7*R* isomer a weak lemon-like odor (detection thresholds 3 and more than 70 $\mu g\ L^{-1}$, respectively), whereas the other isomers are odorless (Acree and others 1985). The methyl esters may be converted to the free acids with either basic hydrolysis or incubation with commercially available esterases. Enantiomers of JA and 12-oxo-phytodienoic acid (OPDA) can be resolved as their methyl esters using cyclodextrin stationary phases in gas chromatography (Wang and others 1996).

Jasmonates are synthesized from α-linolenic acid via 12-oxophytodienoic acid (OPDA). Initial steps in JA biosynthesis catalyzed by a 13-lipoxygenase, an allene oxide synthase (AOS), and an allene oxide cyclase (AOC), are localized within the chloroplast, whereas the final steps including reduction of OPDA and β-oxidation of the carboxylic acid side chain occur in peroxisomes (Strassner and others 2002; Feussner and Wasternack 2002; Schaller and others this issue). Jasmonate concentrations are tightly regulated in plants; the relatively basal amount of each jasmonate family member may differ from tissue to tissue. In the case of mechanical wounding, rapid jasmonate biosynthesis occurs with differential control over the concentration of each compound (Stintzi and others 2001). One of the remarkable features of the jasmonate pathway is that its members have different biological activities. Whereas JA synthesis is required for male fertility in the model plant *Arabidopsis*, its precursor OPDA can play an important regulatory role in defense along with JA (Stintzi and others 2001). Another remarkable feature of the jasmonate pathway is that the activity of some of its members is not confined within the plant. Volatile jasmonate family members, such as the JA metabolite *cis*-jasmone (cJ), help regulate the behavior of some insects-for example, repelling herbivorous species and attracting their predators- and may act as an indicator of JA metabolism in damaged leaves, thereby signaling leaf quality to herbivores. Some evidence suggests that certain volatiles of the jasmonate pathway may enable communication among plants (Birkett and others 2000).

Exogenously applied or intracellularly generated JA following stresses such as wounding, pathogen infection or osmotic stress, induces the expression of numerous genes. Upon wounding, the expression of large numbers of genes is increased or decreased in a jasmonate-dependent manner, and those that are increased include front-line defense-related genes encoding wound-related and pathogenesis-related proteins. Among them are those encoding proteinase inhibitors (PINs) of tomato (Ryan 2000) (Peña-Cortés and others 1988), defensins or thionins of *Arabidopsis* (Thomma and others 1998; Bohlmann and others 1998), and a 23 kDa protein (jasmonate-induced protein, JIP-23) of barley whose function is so far unknown (Hause and others 1999).

In the last decade several jasmonate signaling pathways were described. Among them the wound-response pathway has been studied in detail, particularly in tomato and potato (Peña-Cortés and Willmitzer 1995). JA as well as the 18 amino acid peptide systemin are essential signals at least of a local wound response. Systemin and its precursor protein are upstream-located components of a wound-induced, intercellular signaling pathway that requires both the biosynthesis and action of JA. Systemin is processed from the 200 amino acid peptide prosystemin which is constitutively expressed in vascular bundles (Jacinto and others 1997). The binding of systemin to its membrane-located receptor (Scheer and others 2002) is followed by rapid signaling events including altered levels of cytosolic calcium or the activation of mitogen-activated protein kinases (Stratmann 2003), and leads to the expression of 'early genes'. Concomitantly, a systemin-dependent activation of AOC occurring upon substrate generation may result in a rapid increase of JA, which is known to induce the expression of prosystemin (Ryan 2000; Stenzel and others 2003). Consequently, an amplification in wound signaling by a systemin-dependent AOC activation and by JA-inducible prosystemin gene expression may occur in vascular bundles, where both processes are located (Jacinto and others 1997; Hause and others 2003). However,

the expression of *pin* genes takes place in spongy and palisade parenchyma (Narvaez and others 1993). Based on these data, a signal transfer from the phloem to the mesophyll cells was proposed (Ryan and Moura 2002). Jasmonates generated in the phloem and released in response to wounding might be readily transported through the apoplast and diffuse to cells surrounding the veins. Here, wound-induced JA may be perceived and cause the expression of PINs. Despite these indications for a role of JA in local wound signaling, it is unknown how and where JA accumulation and perception takes place (Howe 2001; Howe this issue). Although the existence of a JA receptor is reasonable, no such receptor has yet been identified and it is even unknown whether the JA perception and/or the accumulation of jasmonates occur extracellularly or intracellularly. Bucking and others (2004) have recently shown that JA localization differs between plants. The monocotyledonous plant barley shows differences from dicotyledonous plants with respect to JA biosynthesis (Maucher and others 2000) and the function of JA during development of seedlings (Hause and others 1996). Moreover, for barley, different JA-signaling pathways have been proposed, because exogenously applied JA and levels of endogenously formed JA led to the expression of different genes, possibly due to independent perception sites (Lobler and Lee 1998; Kramell and others 2000). The results presented by Bucking and others (2004) clearly demonstrated that following application of JA, Jasmonates accumulate extracellularly in tomato, but intracellularly in barley, accompanied in both plants by complete JA signaling, as indicated by the expression of respective marker genes. With a basic understanding of the biochemistry in place, the task now turns to understanding the proteins and genes that are involved in jasmonate signaling. The jasmonate perception pathway is currently being delineated through the use of gain-of-function and loss-of-function mutants. Protein kinases are implicated in early events of jasmonate signaling (Petersen and others 2000). Selective proteolysis may be important in the regulation of jasmonate-dependent gene expression by contributing to the removal of transcriptional regulators from target genes (Xie and others 1998).

Because exogenous JAs do not target specific cell types and often are administered at nonphysiological concentrations, confirmation of the implicated effects of jasmonate requires a more complete understanding of the physiological function of JA in plant growth and development. This understanding would be facilitated by the identification of JA-signaling mutants in diverse plant species and genetic manipulation of either endogenous JA levels or the signal transduction steps that couple JA production to the physiological response.

Jasmonate in Plant Response to Pathogens

JA-mediated Pathogen Response in *Arabidopsis*

Although JA levels in unwounded plant leaves are low, they increase upon wounding independent on plant species (Bell and others 1995; Peña-Cortés and others 1995; McConn and others 1997). However, when one begins to compare the oxylipin profile between *Arabidopsis* and other species and tissues, a complex picture begins to emerge. With other species, JA levels are highest in young growing tissue and in flowers (Creelman and Mullet 1995; Hause and others 2000). Quantitation of oxylipins in unwounded and wounded leaves revealed complex differences between *Arabidopsis* and potato (Weber and others 1997). For example, levels of OPDA were significantly greater than JA in unwounded *Arabidopsis* leaves yet in potato the levels of these two compounds were roughly equivalent. In wounded *Arabidopsis* leaves, JA levels were significantly higher than OPDA levels, yet in wounded potato leaves OPDA levels were higher than JA (Weber and others 1997). In tomato, a similar complex situation exists between leaves and flowers (Hause and others 2000). In tomato flower pistil, the level of OPDA is much higher than JA, yet in flower stalks JA levels are greater than OPDA (Hause and others 2000). Additionally, many oxylipins are generated from more than one fatty-acid substrate resulting in the generation of structurally related molecules that carry one or more functional oxygen-containing groups (for example, keto groups, hydroxyl groups and so on) (Feussner and Wasternack 2002; Howe and Schilmiller 2002). These oxylipins and other lipid-oxidation products produced in plants that are under attack by pathogens or herbivores are thus chemically diverse, suggesting that these compounds have several roles. Indeed, it is very likely that many oxylipins have roles as antimicrobial or anti-insect compounds. Some oxylipins, in particular members of the jasmonate family, however, are potent regulators of defense (Feussner and Wasternack 2002; Howe and Schilmille 2002; Turner and others 2002; Liechti and Farmer 2002, Farmer and other 2003) and fertility (Weber 2002; Li and others 2002; Turner and others

2002). Thus, different species, tissues and stresses may show different oxylipin profiles. More work is needed to characterize how oxylipin profiles differ in different plant tissues, how development and stress may influence these profiles, and to understand how these changes modulate plant growth, development and pathogen response.

Based on the findings that some defense genes are inducible by JAs, it has been speculated that JAs are involved in the pathogen response. The potential of jasmonates to protect plants from infection by pathogens has been shown for the interactions between potato and *Phytophthora infestans* (Cohen and others 1993), between cotton and *Verticillium dahliae*, and between *Arabidopsis* and different pathogens (see reviews Howe and Schilmiller 2002; Weber 2002; Farmer and others 2003;). Endogenous JA levels increase after treatment with the necrotrophic fungus *Alternaria brassicicola* (Penninckx and others 1996). Additionally, biosynthesis as well as signaling mutants are more sensitive to attack by the necrotrophic pathogens *Pythium* and *A. brassicicola*. This was observed with *Pythium* for *coi1, jar1* and *fad3-2fad7-1fad8* (Staswick and others 1998; Vijayan and others 1998), and with *A. brassicicola*, as well as with the necrotrophic bacterium *Erwinia carotovora* for *coi1* (Thomma and others 1998; Norman-Setterblad and others 2000). In contrast, *coi1* and *mpk4* show higher resistance to a virulent strain of the biotrophic bacterial pathogen *Pseudomonas syringae* (Petersen and others 2000; Kloek and others 2001). *Mpk4* is also more resistant to a virulent isolate of the biotrophic oomycete *Peronospora parasitica*, whereas *coi1* shows no difference in susceptibility to a different strain of *P. parasitica* (Thomma and others 1998). For both mutants the salicylic acid (SA)-dependent pathway is a major factor contributing to this resistance. This pathway is hyperactivated in *coi1* upon challenge with *P. syringae* and constitutively active in *mpk4*. To dissect the contribution of the JA and SA pathways, the authors tested *coi1* and *mpk4* plants expressing *NahG*. Investigation of *coi1 NahG* plants revealed that the lower bacterial growth in *coi1* is dependent on the SA pathway whereas the lack of disease symptom development is determined by an SA-independent pathway. The phenotype of *mpk4NahG* plants shows that the lack of defensin and thionin induction is independent of the SA pathway. Based on the data described above, the existence of different pathways involved in the response to necrotrophic pathogens on one hand and biotrophic pathogens on the other hand has been suggested (Thomma and others 2001; Pozo and others this issue). In disagreement with these proposed pathways, the *cev1* mutant is more resistant to the biotrophic fungal pathogen powdery mildew (Ellis and Turner 2001). Due to the variety of different pathogens tested on different mutants, the results are difficult to reconcile with a general model. Taking these results together, a defect in JA sensitivity or biosynthesis increases the susceptibility to necrotrophic bacterial or fungal pathogens and reduces the susceptibility to biotrophic virulent bacterial pathogens, while activation of the JA pathway reduces the susceptibility to necrotrophic pathogens and biotrophic fungal pathogens (*cev1*). Thus, the regulation of resistance/susceptibility of the plant by JA-dependent signaling pathways is determined by the type of pathogen as well as the type of pathogenicity. JA-signaling mutants have also been used to investigate the signal transduction pathways involved in induced systemic resistance (ISR). Growth of *Arabidopsis* plants in soil containing the rhizobacterium *Pseudomonas fluorescens* strain WCS417r results in plants that are less susceptible to infection with virulent strains of fungi and bacteria. Because this ISR is impaired in the *jar1* and *etr1* mutants but not in *NahG* plants, these phenotypes seem to be mediated through a JA and ethylene-dependent but SA-independent pathway (Pieterse and van Loon 1998; Pozo and others this issue).

JA-Mediated Response in Tomato and Grapevine

In Solanaceae, the wound signaling pathway triggers defense mechanisms that usually act against herbivores, but in some cases they can be effective against pathogens as well (Bostock 1999). Both JA and ethylene have been reported to be required for the induction of a defense response in *Arabidopsis* toward *Botrytis cinerea* (for review, see Thomma and others 2001; Pozo and others this issue). Both compounds are required for the development of the wound response in tomato and other Solanaceous species, although the role of ethylene in the induction of wound-responsive genes is still obscure. Wounding results in the cleavage of prosystemin into systemin, which is believed to transduce the wound signal via JA and OGAs, eventually resulting in the onset of defense genes like proteinase inhibitor I (for review, see Ryan 2000; Howe this issue). Because *B. cinerea* expresses endoPG genes during pathogenesis (ten Have and others 1998, 2001), one can assume that the release of OGAs by *B. cinerea* endoPG activity might induce the wound-signaling response of tomato. Testing the jasmonate-deficient mutant, *def1* (*Defenseless*) or *JL5*

(Howe and others 1996), as well as transgenic plants that overexpress prosystemin, designated PSoe (McGurl and others, 1994), or that have reduced levels of prosystemin by antisense expression, designated PSas (McGurl and others 1992), has provided information on JA involvement in pathogen response in tomato plants. The prosystemin-overexpressing line presents a constitutive wound/herbivore defense response, whereas the *def1* mutant and the prosystemin antisense line are impaired in such a response. Both *def1* and the prosystemin antisense line were more susceptible to *B. cinerea* than the wild-type progenitor tomato cv Castlemart, whereas the prosystemin-overexpressing line was highly resistant (Li and others (2002). The precise role of OGAs in determining the outcome of the interaction between *B. cinerea* and tomato remains to be clarified. Recently, Audenaert and others (2002) reported that the mutant *def1* did not show increased susceptibility to *B. cinerea*, which seems to contradict the results of Diaz and others (2002). The explanation for such a difference is in how these experiments were conducted. In one case, detached leaves from plants that were considerably older than the plants used by Diaz and others (2002 were used. In *Arabidopsis*, JA and ethylene are considered to act synergistically in the response to pathogens (Thomma and others 1999). In tomato; however, these signals seem to act in an independent manner. Ethylene pretreatment of mutant lines, altered in JA or systemin signaling, as well as the wild-type tomato cv Castlemart, consistently resulted in a similar increase of resistance.

Very recently, Repka and others (2004) showed that treatment with methyl jasmonate (MeJA) stimulates a multicomponent defense response in leaves and suspension-cultured cells of grapevine (*Vitis vinifera* L. cv. Limberger). MeJA induces development of necrotic lesions similar to that normally associated with resistance to avirulent pathogens. Sustained exposure of both leaves and cell-suspension cultures to MeJA provoked hypersensitive cell death, stimulated medium alkalinization accompanied by massive callose deposition, but did not induce accumulation of hydrogen peroxide from the oxidative burst. MeJA elicits expression of the *PR-1*, *PR-2*, and *PR-3* genes, which are also induced after infection by various pathogens and by treatment with SA or its analogs (Yang and others 1997; Repka 2001). After several days systemic accumulation of a large number of defense-associated proteins, including pathogenesis-related proteins, peroxidase, cell wall extensin and enzymes involved in the phenylpropanoid biosynthetic pathway was induced. Exogenously supplied MeJA

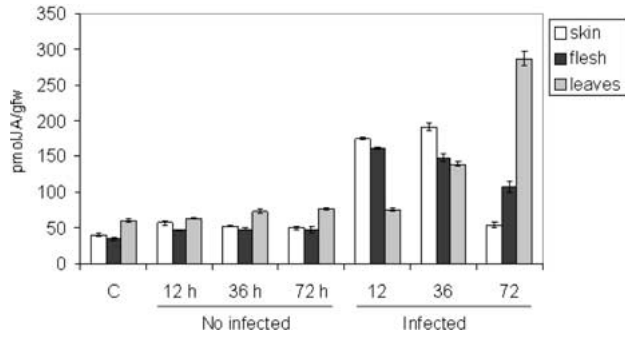

Figure 1. Endogenous jasmonic acid levels in grapevine berries. Leaves and mature grape berries of Thompson seedless were infected with a spore suspension (1×10^6 per ml) of *Botrytis cinerea* and samples were collected at different times from control (no infected) and from infected plants (infected) after inoculation. The infected tissue was kept at 25°C with a 16/8 h photoperiod, before harvesting for time course analysis of the infection between 0 and 72 hrs. Skin and flesh were separated and frozen in liquid nitrogen before JA determination according to Barrios and others (2004). As controls, leaves and mature fruits were mock-inoculated, according to the procedure used in each case.

resulted in the production of large quantities of SA. Free and conjugated SA were present in a ratio similar to that described in the HR to TMV (Malamy and Klessig 1992). Whether SA produced upon MeJA treatment is the endogenous signal for defense gene induction remains, however, to be elucidated. These cumulative results suggest that grapevine cells that perceived MeJA generated a cascade of events acting at both local and long distances, and causing the sequential and coordinated expression of specific defense responses with a timing and magnitude similar to the typical hypersensitive response against pathogens.

Accordingly with these data, preliminary results in our group show that grapevine tissues respond to infection with *B. cinerea* by accumulating JA in a different form, depending on the affected tissue. Thus, an increase of JA levels in infected leaves correlates with the formation of necrotic lesions which takes place 72 hours post-infection, whereas in grape fruit the accumulation of JA occurs before lesion or infection symptoms are observed. This accumulation reaches its maximum between 12 and 36 hours after inoculation (Figure 1). More interestingly, exogenous application of jasmonate to detached mature grape fruit decreases the damage caused by *B. cinerea* infection but has no effect in protecting grape leaves (H. Peña-Cortés un published results). The protective effect of jasmonate was observed when the treatment occurred at room

temperature whereas no effects was observed if the grapes were stored under low temperature (<4 °C). Thus, the protective effect of jasmonate seems to be temperature dependent suggesting either that some steps of the grape jasmonate perception pathway or that the JA-mediated activation of the defense mechanism is inhibited by low temperature. These results suggest that jasmonate is involved in the defense mechanism against *B. cinerea* in the grape fruit but not in grapevine leaves.

Information has been reported related to the use of jasmonate for controlling pathogen disease in postharvest. Some fruits, like strawberry, mangoes or grapevine are produced in one place and are stored or transported for several days before reaching the market. To avoid pathogen disease, especially those caused by fungi, such fruits are treated with certain substances or maintained under refrigeration and modified atmospheres. It has been reported that application of MeJA vapor for 24 hours at 20°C to strawberries controlled *B. cinerea*, a major fungal disease of postharvest fruits and vegetables, for up to 14 days in storage. Methyl jasmonate not only reduced the mold but it enhanced the flavor of the strawberries as well. The treatment did not affect the firmness of the fruit. Similar effects have also been reported for grapes (Stanley 1998). More recently, Artes-Hernandez and others (2002) reported the effect of jasmonate combined with a modified atmosphere in the conservation and transport over 7 weeks at 0°C of the seedless grape cv "Autumn Seedless". The grapes treated with jasmonate presented an aspect, flavor and texture superior to untreated grape clusters. Thus, it seems that if the right amount of jasmonate is used under proper storage conditions it may be a practical treatment to ensure the safety of many fresh-cut and whole fruits and vegetables.

Jasmonate in Plant Response to Insects

Resistance or tolerance of plants to insect herbivores and pathogens is mediated via constitutive or induced defense mechanisms (Mauricio and others 1997; Buell 1998). Inducible defenses play a major role in conferring disease resistance against plant pathogens (Maleck and Dietrich 1999), and their effects on phytophagous insects can include increased toxicity, delay of larval development, or increased attack by insect parasitoids (Baldwin and Preston 1999). Resistance against herbivorous insects and some fungal pathogens depends on wound-response signaling via JA and ethylene (Maleck and Dietrich 1999). The common action of JA and ethylene in wound signaling was demonstrated initially with tomato plants (O'Donnell and others 1996). Herbivory causes an increase in endogenous jasmonic acid, exogenous JA induces a similar though not identical set of compounds as insect herbivory, and the compounds induced by herbivores and JA acid have been linked causally to herbivore performance (Halitschke and Baldwin this issue). JA acid has not been found to be directly toxic to herbivores; MeJA that was incorporated into an artificial diet and fed to *Trichoplusia ni* and *Manduca sexta* larvae did not affect larval growth (Avdiushko and others 1997). Herbivore damage to plants, including tomato and *Arabidopsis*, can cause increases in endogenous JA levels (Doares and others 1995; McConn and others 1997).

In essence, tissue damage caused by insect feeding activates an octadecanoid signaling cascade that culminates in JA biosynthesis and production of antifeedant proteinase inhibitors (PIs; Broadway and others 1986) and other putative defense molecules. Considerable evidence implicates induced changes in phytochemical content in causing an increase in resistance of tomato foliage to herbivores (Broadway and others 1986; Johnson and others 1989; Thaler and others 1996). For example, *Spodoptera exigua* (beet armyworm) fed on foliage from plants damaged previously by *H. zea* experience lower growth rates and survival than larvae feeding on undamaged control plants (Stout and Duffey 1996). Beet armyworm fed on foliage from plants sprayed previously with JA acid also experienced a lower growth rate than larvae on unsprayed control plants in the laboratory (Thaler and others 1996). Further evidence that jasmonate inducible compounds play a role in induced resistance comes from transgenic tomato plants that contained an antisense prosystemin gene that prevents systemic signaling for the *novo* synthesis of proteinase inhibitor and polyphenol oxidase (Orozco-Cardenas and others 1993). Larval *Manduca sexta* had higher growth rates on these transgenic plants than insects feeding on untransformed plants that induce proteinase inhibitor and polyphenol oxidase following herbivory (Orozco-Cardenas and others 1993). Finally, proteinase inhibitor and polyphenol oxidase, compounds induced by *H. zea* feeding, reduce the growth of several lepidopteran pests, including beet armyworm and *H. zea*, when incorporated into artificial diets (Broadway and Duffey 1988; Felton and others 1989). Mutations that reduce JA production result in increased susceptibility to herbivores. For example, a tomato mutant unable to convert 13-hydroperoxylinolinic acid into 12-oxophytodienoic acid, *def1*, does not accumulate PIs in

response to wounding and is significantly more susceptible to tobacco hornworm than wild-type plants (Howe and others 1996). Similarly, an *Arabidopsis* triple mutant (*fad3-2 fad7-2 fad8*) also lacks wound induced JA biosynthesis, and as a consequence is more susceptible to fungal gnats (McConn and others 1997).

The recent identification and characterization of the *opr3* mutant (Sanders and others 2000; Stintzi and Browse, 2000) has helped to demonstrate that resistance to insect and fungal attack can be observed in the absence of JA. The *Arabidopsis opr3* mutant is defective in the isoform of 12-oxophytodienoate (OPDA) reductase required for JA biosynthesis. Oxylipin signatures of wounded *opr3* leaves revealed the absence of detectable 3*R*, 7*S*-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. The authors 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 (Stintzi and others 2001).

The known roles of the jasmonate pathway in resistance phenomena have recently been extended. *Def1* tomato plants, which are deficient in JA, are highly susceptible to two-spotted spider mites (Li and others 2002). The decreased resistance of *def-1* plants was correlated with reduced JA accumulation and expression of defensive proteinase inhibitor (PI) genes, which were induced in mite-damaged wild-type leaves. Treatment of *def-1* plants with methyl-JA restored resistance to spider mite feeding and reduced the fecundity of female mites. In contrast to this, the authors show that plants expressing a *35S::prosystemin* transgene that constitutively activates the octadecanoid pathway in a *Def-1* dependent manner were highly resistant to attack by spider mites and western flower thrips (*Frankliniella occidentalis*), another cell-content feeder of economic importance. Thus, Li and others (2002) claim that these findings indicate that activation of the octadecanoid signaling pathway promotes resistance of tomato to a broad spectrum of herbivores. The resistance of the transgenic lines expressing the 35::prosystemin transgene to attack by spider or thrips may result from either higher accumulation of JA or some of its precursor, but such analysis is not yet complete.

Increasing evidence indicates that JA and related signaling molecules play an important role in regulating volatile-mediated plant defenses against two-spotted spider mite (Dicke and others 1990, 1999; Arimura and others 2000; Ozawa and others 2000). In lima bean plants JA-induced volatile blends are similar to those induced by spider mites. However, predatory mites prefer plants that are attacked by spider mites to chemically induced plants when given the choice (Dicke and others 1999). Thus, in addition to JA, there are insect-specific signals leading to predator attraction. By contrast, JA-related defense pathways appear to be sufficient to reduce insect herbivory by increasing caterpillar parasitism in the field (Thaler 1999), suggesting that JA is a major, but not the only component of induced defenses. Nevertheless, spider mites cause lima bean plants to emit significant amounts of methyl-SA, in addition to JA-related volatiles (Dicke and others 1999), suggesting that both signaling pathways operate together in that species. Perhaps the balance between different signaling pathways adjusts defense characteristics against particular insects or pathogens.

Recently, Omer and others (2002) reported that exogenous jasmonate promotes host plant resistance to spider mite in grapevine plants. Foliar JA application at concentrations that caused no phytotoxicity significantly reduced the performance of both herbivores Pacific spider mite, *Tetranychus pacificus* McGregor (Acari: Tetranychidae), as well as the root-feeding grape phylloxera *Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae). There were less than half as many eggs produced by spider mites feeding on the induced leaves compared with control grapevine leaves. Induction reduced the numbers of phylloxera eggs and nymphal instars by approximately threefold and twofold, respectively, on induced compared with control grapevine roots. The negative demographic effects of JA application appeared to be caused by changes in fecundity of the Pacific spider mite, and possibly changes in development rate and fecundity of grape phylloxera. These results together suggest that exogenous application of jasmonate seems to be enough for decreasing spider mite performance in tomato (Li and others 2002) and grapevine plants (Omer and others 2002).

Transgenic Approaches to Modulate JA Biosynthesis

To modulate endogenous JA levels, construction of transgenic plants either overexpressing or inhibiting (RNA antisense) a gene encoding enzymes of the JA biosynthetic pathway has been performed. So far, these approaches have only been partially successful. Most of the effort in *Arabidopsis*, tobacco and tomato plants such as exhibiting reduced expression of At*LOX2* (Bell and others 1995) or overexpressing AOS and AOC, respectively, do not show changes in the basal levels of JAs (Laudert and others 2000; Wang and others 1999; Stenzel and others 2003; Barrios and others 2004). However, there are alterations in JA accumulation after wounding in these approaches. In the case of AOS, three different effects were found: (1) Constitutive overexpression of a AOS in tobacco, *Arabidopsis* (Schaller, 2001) or in tomato (Barrios and others 2004) led to elevated jasmonate levels only upon wounding, which suggests a lack of AOS substrate in nonwounded tissues. (2) Constitutive overexpression of the AOS from flax in potato revealed 6- to 12-fold higher levels of JA without constitutive expression of JA-responsive genes, which suggests sequestration of elevated JA (Harms and others 1995). Upon wounding, the expected JA-/wound-responsive gene expression occurred, but in addition, a rise in the levels of JAs was observed (Harms and others 1995). (3) Inducible expression of flax-AOS, lacking the chloroplast transit sequences, in tobacco led to an increase of JA level upon wounding (Wang and others 1999). Similarly, overexpression of AOC does not affect the basal levels of JA in tomato leaves (Stenzel and others 2003). These data indicate that in a plant leaf the generation and activity of the stress signal JA may depend on spatial and temporal expression of its biosynthetic genes, on intracellular compartmentation, and on basal and induced levels of the respective substrates. To date, the behavior of these types of transgenic plants against insects or pathogens has not been analyzed.

There are several possible reasons for the lack of the expected phenotype in transgenic approaches: one of the difficulties in altering JA biosynthesis might be caused by the redundancy of components of the biosynthetic pathways and the existence of several homologous proteins for some of these enzymes. Six sequences with homology to lipoxygenases and four genes encoding putative allene oxide cyclases have been found in the *Arabidopsis* genome, whereas AOS and the reductase converting OPDA are each encoded by a single gene. Additionally, the expression of the protein in the wrong compartment could prevent an effect in overexpressing transgenic lines. It might also be possible that the plant counter-regulates the production of higher amounts of these signaling molecules. Therefore, to able to manipulate JA levels, it is necessary to know more about the regulation of JA biosynthesis. The functional significance of these genes for insect resistance is uncertain.

Other studies have demonstrated the involvement of the oxylipins pathway in response to insects. Thus, antisense depletion of potato *LOX-H3* mRNA leads to reduced accumulation of antifeedant PIs and greater susceptibility to polyphagous insects without influencing JA biosynthesis (Royo and others 1999). Cosuppression experiments suggest that LOX2 contributes to wound-induced JA biosynthesis that affects downstream genes, such as *VSP* (Bell and othersy1995). Thus *Arabidopsis* LOX2 may also influence insect herbivory. Antisense-mediated HPL depletion in transgenic potato plants has identified this enzyme as a major route of 13-fatty acid hydroperoxide degradation in the leaves. Although these transgenic plants have highly reduced levels of both hexanal and 3-hexenal, they show no phenotypic differences compared with wild-type, particularly in regard to the expression of wound-induced genes. However, aphids feeding on the HPL-depleted plants display approximately a two-fold increase in fecundity above those feeding on nontransformed plants, consistent with the hypothesis that HPL-derived products have a negative impact on aphid performance. Thus, HPL-catalyzed production of C6 aldehydes may be a key step of a built-in resistance mechanism of plants against some sucking insect pests. Overexpression of allene oxide synthase (AOS) in transgenic tomato plants under the control of the 35S cauliflower mosaic virus promoter show basal levels of JA similar to those in non-transformed plants. However, elevated JA levels after mechanical wounding were 1.5 to 2-fold higher in transgenic plants than in wounded control plants. The accumulation of JA occurs faster in the transgenic plants, allowing a faster accumulation of wound-responsive genes. Tomato leaf miner larvae fed on the transgenic leaves showed reduced weight gain (Figure 2A) and oviposition by whiteflies on the transgenic leaves was reduced (Figure 2B). Infection of transformed leaves with the fungal pathogen *B. cinerea* resulted in formation of necrotic lesions that grew at the same rate as in non-transformed leaves. Thus, overexpression of AOS in tomato transgenic plants leads to an increase of wound-induced JA accumulation and to increased resistance to some insect

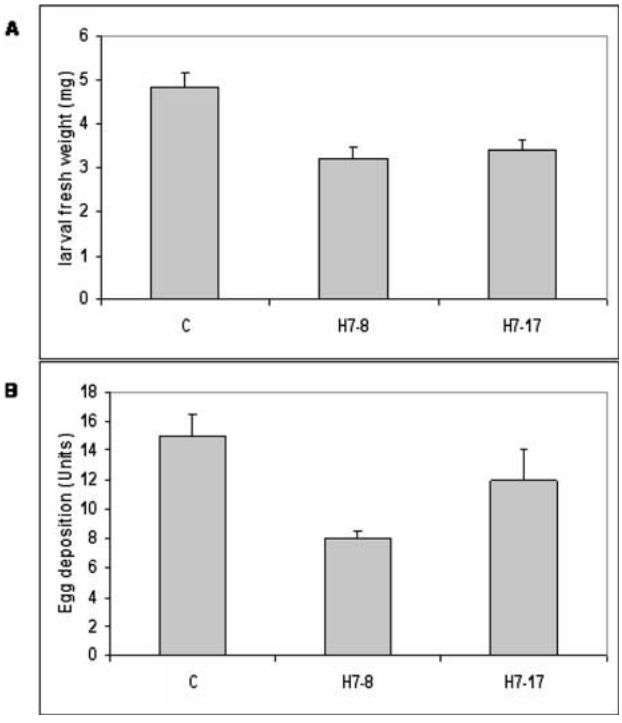

Figure 2. Growth of *Tuta absoluta* larvae and egg deposition of whiteflies on tomato plants (**A**) First instar larvae of *Tuta absoluta* were fed on non-transformed tomato leaves (**C**) or on leaves from transgenic tomato lines (H7-8,-17) for 14 days. All experiments were performed by using detached leaves from 9-10 plants per genotype. The experiment was repeated three times with similar results. Larval weight values are mean ± SD (n = 18). (**B**) Mixed populations of non-transformed tomato plants (**C**) with transgenic AOS tomato (H7-8; H7-17) plants (6 weeks old) were placed in a net enclosure and subjected to infestation with *T. vaporarium* adults. Data are given in egg units per plant 48 hours after infestation, and represent three independent experiments with the average ± SD (bars) egg number for 10 plants each of C, H7-8 and H7-17.

pests (Barrios and others 2004). Although these plants showed higher levels of JA than the non-transformed ones following infection with *B. cinerea* no differences are observed in disease symptoms. This result is in agreement with the data reported by Diaz and others (2002) suggesting that increased endogenous levels of JA are not involved in tomato response to this necrotrophic pathogen.

At the end of the biosynthetic JA pathway, JA is catabolized further to form its volatile counterpart MeJA by a S-adenosyl-L-methionine: jasmonic acid carboxyl methyltransferase (JMT). In an attempt to alter JA metabolism, a jasmonic acid carboxyl methyltransferase (JMT) was overexpressed and led to increased levels of methyl jasmonate but unaltered levels of JA (Seo and others 2001). In the transgenic *Arabidopsis* overproducing the *JMT* gene, various jasmonate-responsive genes were constitutively expressed in the absence of wounding or jasmonate treatment. This finding suggests that MeJA formation is a critical control point for jasmonate-regulated plant responses. Importance of *JMT* gene activation in the MeJA-induced defense response was demonstrated by the illustration that the transgenic *Arabidopsis* exhibited enhanced resistance to the virulent fungal pathogen *B. cinerea* (Seo and others 2001). Further characterization of these plants has demonstrated a constitutively expression of various defense genes and an enhanced resistance to a bacterial pathogen, *Pseudomonas syringae* pv *tomato* DC3000 (Jung and others 2003). These results indicate that MeJA mediates plant defense responses against a broad spectrum of pathogens including fungi and bacteria. Thus, genetic introduction of the MeJA-producing gene could be a way to achieve fortified disease resistance in plants.

Recently, Kessler and others (2004) used transformed lines of the wild tobacco species *N. attenuata*, which express *N. attenuata* lipoxygenase 3 *(NaLOX3)*, hydroperoxide lyase (*NaHPL*), and allene oxide synthase (*NaAOS*) in an antisense orientation (as*lox*, as-*hpl*, as-*aos*, respectively) to study herbivore-induced plant responses in nature. Initial field experiments have shown that the three transformed plant lines (as-*lox*, as-*aos*, as-*hpl*) have similar characteristics in the field and in the laboratory (Halitschke and Baldwin 2003) and differ in their responsiveness to *M. sexta* attack. The authors found dramatically more herbivore damage on as-*lox* plants than on WT, as-*hpl*, or as-*aos* plants, which corresponds to the increased performance of *M. sexta* caterpillars on as-*lox* plants. Similarly, herbivores attacked a significantly greater proportion of as-*lox* plants compared to all other lines in the experimental population. Although herbivory was equally distributed among WT, as-*aos*, and as-*hpl* plants, it was significantly higher on plants with strongly attenuated induced responses (as-*lox*), which suggests that a plant's ability to elicit defenses influences the distribution of herbivory within a plant population.

Plant Development and Fruit Ripening

The role of JA in flower development has been well documented by using several *Arabidopsis* mutants (Feys and others 1994; Xie and others 1998; Xu and others 2002; Devoto and others 2002). Reproduc-

tive dysfunction in JA-deficient *Arabidopsis* plants results from a combination of defects in anther filament elongation, anther dehiscence, and pollen maturation (Sanders and others 2000; Stintzi and Browse 2000; Ishiguro and others 2001; Park and others 2002). In contrast to JA signaling mutants of *Arabidopsis*, reciprocal crosses in tomato plants showed that *jai1* plants are male fertile and female sterile (Li and others 2001). This observation suggested that JA plays different roles in the reproductive development of different plant species. On the other hand, several defense-related phenotypes of *jai1* plants are similar to those of *Arabidopsis coi1* mutants, including loss of expression of defense-related genes in response to wounding and MeJA, insensitivity to the phytotoxin coronatine (COR), and increased resistance to virulent strains of *Pseudomonas syringae* (Li and others 2001; Zhao and others 2003). Recently the tomato mutant *jai1* was identified as the homolog of *coi1* (Li and others 2004). In contrast to *coi1*, the *jai1* plants are affected in seed maturation and glandular trichome development (Li and others 2004; Howe this issue). The molecular mechanism by which JA promotes male reproductive development remains unknown. Recent studies support the hypothesis that JA controls the expression of genes that are required for normal anther development and pollen maturation (Mandaokar and others 2003).

Although jasmonate was detected in plant fruits many years ago its involvement in fruit development and/or ripening has not been as well studied as its involvement in flower development. Jasmonate is present in a variety of plant organs, with the latest amounts in fruit parts (Meyer and others 1984) including young "Golden Delicious" apples (Fan and others 1997). Exogenous jasmonate applications promote climacteric fruit ripening by positively or negatively influencing ethylene production, depending on fruit development (Saniewski and others 1986), accelerating chlorophyll degradation and β-carotene accumulation in tomato (Saniewski and Czapski 1983; Perez and others 1993), and promoting ripening-related aroma compounds (Fan and others 1997b). Exogenous applications of jasmonate accelerate degreening and stimulate ethylene production in apple fruit in a concentration- and developmental stage-dependent manner (Fan and others 1997b, 1998). Additionaly, endogenous jasmonate concentrations rise coincident with the onset of ripening in apple and tomato fruit (Fan and others 1998). Recently, it has been shown that exogenous application of jasmonate enhanced the rate of fruit ripening in peach 'Redhaven' (Janoudi and Flore 2003), and increased ethylene production at the climacteric stage in mangoes (Lalel and others 2003). Skin color of ripe fruit was significantly improved with exogenous application of methyl jasmonate as well as an increase in the amount of fatty acids, total aroma volatiles, monoterpenes, sesquiterpenes, aromatics, norisoprenoids, alcohols and esters in the pulp of fruit. In general, exogenous application of MeJA significantly promoted biosynthesis of ethylene, fatty acids and ripening and aroma volatile compounds during fruit ripening. ransient increases in endogenous JA and MeJA levels also occurred during the onset of tomato fruit ripening (Fan and others 1998). The transient increase in levels of jasmonates occurring at the early state of tomato and apple fruit ripening is detected prior to the rapid rise of ethylene production. This increase occurs simultaneously with the initial detection of ethylene Tbiosynthesis, indicating jasmonates may interact with the ethylene signal (Fan and others 1998). Together all these results suggest that jasmonates are involved at the early steps in the modulation of climacteric fruit ripening and might be required for triggering ethylene production. More interestingly, in non-climacteric fruit with low ethylene production, the endogenous amount of JAs is high at the beginning of fruit development and decreases toward maturation. Indeed, JA and MeJA are mainly present in the very early stage of seeded grape berries (Kondo and Fokuda 2001), and their changes were similar to those of JA in sweet cherry fruit (Kondo and others 2000) and MeJA in strawberry fruit (Gansser and others 1997). The changes of endogenous jasmonates in climacteric apples and in nonclimacteric fruits demonstrate that jasmonates may influence cell division because their levels were higher at the beginning of fruit development. JAs may also affect aroma formation and further events during fruit development. However, the mechanism of how jasmonates affect fruit development and fruit ripening either in climacteric or in non-climacteric fruits remains unknown. Jasmonates are a prime example of aroma compounds that are also extremely potent phytoeffectors. Several works reported parallels between the time course of their endogenous levels in developing fruits and other events during development. It is possible that endogenous jasmonates directly influence these events. However, their respective phytoeffector-like actions have previously been investigated by an exogenous addition, mostly of a mixture of synthetic isomers. Further studies will be necessary to determine whether results obtained from exogenous application can be transferred to endogenous jasmonate stereoisomers and how they

interact with other effectors (hormones) in the fruit processes.

Perspectives of biotechnological Uses of Jasmonate

Abundant evidence has demonstrated that the jasmonate pathway plays a crucial role not only in defense against notorious pests or pathogens but also in plant development. Because many of the effects observed following exogenous application of JAs to plant tissues are ,being corroborated by using either mutant plants or transgenic approaches and considering the results obtained in fruit crops like grapevine, strawberry or mangoes after JAs treatment during fruit development or postharvest, we support the potential of these substances for use in agricultural practices. In addition to the well-documented evidence demonstrating a role of JAs in plant protection against bacteria and fungi, it has also become clear that induced resistance to herbivores in many, if not all, plants is mediated by the jasmonate pathway. We now know that the JA pathway plays important roles in defense against not only insects but also against at least one other class of arthropod, *Arachnida*. Spider mites, and other organisms like grape phylloxera, have proven to be difficult pests to control using conventional methods as reported by Omer and others (2000), in greenhouse experimental treatments with JAs of these pests. Because greenhouse experiments tend to be predictive of field results with jasmonic acid (Thaler 1999), these results suggest that field trials are warranted in the hope of using induced resistance for pest management in mainstream agriculture.

Controlling fruit ripening is a challenge faced daily by growers, shippers and retailers of all types of fruits. Usually the objective is to slow the process to increase the marketable period or "shelf-life", but getting ripening off to a rolling start can also be sticky. Ethylene gas produced naturally by many fruits including apples, bananas, kiwis and mangoes starts the ripening process, but what starts ethylene production is unclear. We now know that another gaseous compound, MeJA, is produced naturally by climacteric and non-climacteric fruits. Furthermore, MeJA and JA also induce ethylene production in tomatoes and apples. This results in earlier ripening, including enhanced aroma production in the climacteric apple fruit, but can also occur in nonclimacteric fruit like grape or strawberry. Thus, JAs might play an important role in fruit development and ripening in both climacteric and nonclimacteric fruits.

Many aspects related to JAs remain unknown and should add considerably to interest in the jasmonate pathway. For instance (1) more information on jasmonate perception will provide vital knowledge on many aspects of regulation; (2) improved understanding of cell biology of jasmonates will explain how these compounds are transported within and between cells and tissues to reach their sites of action; (3) more detailed knowledge about how the jasmonate pathway contributes to the coordination of direct defense responses (that is, regulation of defense genes within the plant) and indirect defense responses.(for example, control of the behavior of predatory insects in the plant's environment) is needed; (5) identification of target genes regulated by different jasmonates will help us to understand how these molecules exert their multiple remarkable biological effects; (6) progress on JAs' effects in fruit development and ripening will help explain the real role that these substances in climacteric and non-climacteric fruits are playing. Finally, further investigation of gas-phase signaling by volatile methyl jasmonate (MeJA) within the plant may contribute to the general knowledge of the biology of volatile regulators of gene expression. Answering such basic scientific questions and advances in the manipulation of the pathway hold promise for future strategies in agriculture.

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