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Coi1-Dependent Signaling Pathway Is Not Required for *Mi-1*–Mediated Potato Aphid Resistance

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Tomato (*Solanum lycopersicum)* has a unique resistance gene, *Mi-1*, that confers resistance to animals from distinct taxa, nematodes, and piercing and sucking insects. *Mi-1* encodes a protein with a nucleotide-binding site and leucine-rich repeat motifs. Early in the potato aphid (*Macrosiphum euphorbiae*)–tomato interactions, aphid feeding induces the expression of the jasmonic acid (JA)-regulated proteinase inhibitor genes, *Pin1* and *Pin2*. The *jai1-1* (*jasmonic acid insensitive 1*) tomato mutant, which is impaired in JA perception, was used to gain additional insight into the JA signaling pathway and its role in the *Mi-1*–mediated aphid resistance. The *jai1-1* mutant has a deletion in the *Coi1* gene that encodes a putative F-box protein. In this study, aphid colonization, survival, and fecundity were compared on wild-type tomato and *jai1-1* mutant. In choice assays, the *jai1-1* mutant showed higher colonization by potato aphids compared with wild-type tomato. In contrast, no-choice assays showed no difference in potato aphid survival or fecundity between *jai1-1* and the wild-type parent. Plants homozygous for *Mi-1* and for the *jai1* mutation were not compromised in resistance to potato aphids, using either choice or no-choice assays. In addition, the accumulation of JA-regulated *Pin1* transcripts after aphid feeding was *Coi1* dependent. Taken together, these data indicate that, although potato aphids activate *Coi1*-dependent defense response in tomato, this response is not required for *Mi-1*–mediated resistance to aphids.

Faced with continual threats from myriad biotic and abiotic agents, plants have evolved a number of strategies to help ensure their own survival. In addition to physical barriers at the tissue surface, plants fight infection using basal defenses and classical gene-for-gene resistance responses. While basal defenses do little to mitigate the growth and multiplication of pests and pathogens, they do limit disease severity. Gene-for-gene resistance is mediated by plant resistance (*R*) genes (Flor 1971) and is initiated via specific recognition of a pathogen-derived avirulence (*avr*) gene product by a host *R* gene product. Both basal defense and *R* gene–mediated defense responses involve pathways regulated by plant hormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene (Hammond-Kosack and Parker 2003; Martin et al. 2003).

The tomato gene *Mi-1* is the first cloned insect resistance gene (Kaloshian 2004). Besides conferring resistance to potato aphid (*Macrosiphum euphorbiae* Thomas), *Mi-1* also confers resistance to three species of root-knot nematodes (*Meloidogyne arenaria* [Neal] Chitwood, *M. incognita* [Kofoid & White] Chitwood, and *M. javanica* [Treub] Chitwood) and two biotypes of whiteflies (*Bemisia tabaci* Gennadius and *B. tabaci* biotype B) (Milligan et al. 1998; Nombela et al. 2003). *Mi-1* was introgressed into cultivated tomato, *Solanum lycopersicum* L., from its wild relative *Solanum peruvianum* L. (Smith 1944). *Mi-1* belongs to the largest class of resistance genes cloned to date (Martin et al. 2003). It encodes a protein with a coiled-coil domain, nucleotide-binding site, and leucine-rich repeat (LRR) motifs (Milligan et al. 1998). In addition to *Mi-1*, nematode and insect resistance in tomato requires the presence of another gene, *Rme1*. *Rme1* was discovered in a genetic screen and appears to be specific for Mi-1 function (Martinez de Ilarduya et al. 2001, 2004). The identity of this gene is still unknown.

Mi-1 appears to mediate distinct resistance mechanisms to nematodes and insects, or the manifestation of resistance is distinct in roots compared with leaves. *Mi-1*–mediated defense responses to nematodes are associated with induction of hypersensitive response (HR) (Dropkin 1969), one of the hallmarks of gene-for-gene resistance. In contrast, no HR is involved in *Mi-1*–mediated resistance to aphids (Martinez de Ilarduya et al. 2003). Aphids maintained on *Mi-1* plants are characterized by reduced feeding, fertility, and survival (Kaloshian et al. 1997, 2000). Starvation and desiccation are the likely causes of death, as symptoms subside when aphids are transferred from resistant to susceptible genotypes (Kaloshian et al. 1997).

Another distinct feature of *Mi-1*–mediated resistance to insects is that resistance is developmentally regulated, with *Mi-1* tomato plants remaining susceptible to insects for up to five weeks (Kaloshian et al. 1995; Pascual et al. 2000). In adult plants, fully expanded leaves are resistant to aphids irrespective of leaf position, while expanding leaves remain aphid-susceptible throughout the life of the plant (Kaloshian et al. 1997). The developmental regulation of *Mi-1* is absent in roots, in which resistance to nematodes is expressed early in development. It is not clear whether the developmental regulation is associated with *Mi-1*. *Mi-1* transcripts are present in leaves and roots early in development, and transcript levels do not change after challenge with either nematode or insect (Martinez de Ilarduya and Kaloshian 2001). Therefore, either *Mi-1* is post-transcriptionally regulated differently in roots and leaves or another component in the resistance signaling pathway is developmentally regulated differentially in roots and leaves.

Similar to plant responses to pathogens, plant defense responses to piercing and sucking insects involve the accumulation of pathogenesis-related (PR) proteins and RNA (Kaloshian

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and Walling 2005; Thompson and Goggin 2006). In the tomato-potato aphid interactions, aphid infestation induces faster and higher levels of *PR-1* transcript accumulation in *Mi-1* tomato, the incompatible interaction, compared with the compatible interaction (Martinez de Ilarduya et al. 2003). In the incompatible *Mi-1* aphid interaction, *PR-1* transcripts were detected as early as 6 h and remained at high levels 48 h after aphid infestations. In general, *PR-1* expression is associated with the SA signaling pathway, although in tomato, *PR-1* transcripts have been shown to accumulate after exogenous application of SA, methyl jasmonate (MeJA), or ethylene (Chao et al. 1999; Li et al. 2006; van Kan et al. 1995). A role for the SA signaling pathway in *Mi-1*–mediated resistance to aphids was identified (Li et al. 2006). Introducing *NahG*, which degrades SA to catechol, in the *Mi-1* background attenuated the resistance to potato aphids. Similarly, root-knot nematode growth was observed in *Agrobacterium rhizogenes* transgenic roots expressing *NahG* in the presence of *Mi-1*, indicating also a role for SA in *Mi-1*–mediated resistance to root-knot nematodes (Branch et al. 2004).

The role of JA and ethylene-regulated defense pathways in *Mi-1*–mediated aphid resistance remains unclear. Although cross talk among SA, JA, and ethylene pathways has been documented, simultaneous activation of plant defenses responsive to these three signal molecules has only recently been documented (Schenk et al. 2000). In tomato, proteinase inhibitors *Pin1* and *Pin2* transcripts accumulated transiently 6 h after aphid infestation in both compatible and incompatible aphid interactions (Martinez de Ilarduya et al. 2003). *Pin1* and *Pin2* are regulated by JA, which is the terminal product of the octadecanoid pathway (Creelman and Mullet 1997; Farmer and Ryan 1992). Therefore, the upregulation of these genes by aphid feeding may implicate the octadecanoid signaling pathway in aphid resistance.

To investigate the role of JA in *Mi-1*–mediated resistance to potato aphids, the well-characterized tomato *jasmonic acid insensitive 1* (*jai1-1*) mutant was used (Li et al. 2001). Although *jai1-1* mutant plants are able to synthesize JA, they are impaired in JA perception (Li et al. 2001). Recently, the *jai1* mutation was cloned and was shown to correspond to mutations in the tomato homolog of the *Arabidopsis COI1* gene, which encodes a protein with a putative N-terminal F-box domain and 16 imperfect LRR (Li et al. 2004; Xie et al. 1998). COI1 is a part of the SCF complex, a multiprotein complex that includes Cullin and Skp1 and functions as an E3-type ubiquitin ligase (Bai et al. 1996). It is postulated that JA is regulated by a repressor that is recruited by the LRR domain of COI1 to the E3 complex and is marked for degradation through ubiquitination by the 26S proteosome (Turner et al. 2002).

The *Coi1*-dependent defense responses to potato aphids were evaluated using the tomato *jai1-1* mutant and its wild-type parent. After introducing the *jai1* mutation in the *Mi-1* background, the requirement for *Coi1* in the *Mi-1*–mediated aphid resistance was also evaluated. Our results indicated no role for the *Coi1*-dependent responses in basal defense or *Mi-1*–mediated resistance to potato aphids in tomato.

RESULTS

The role of JA in basal resistance to potato aphid.

To investigate whether the *Coi1*-dependent signaling plays a role in tomato defense against aphids, the interaction of the potato aphid with *jai1-1* mutant tomato versus its wild-type parent tomato cv. Castlemart (CM) were compared. Since homozygous *jai1-1* plants are sterile (Li et al. 2004), the mutation is maintained in heterozygous state. To obtain homozygous *jai1-1* plants, germinating seeds segregating for this locus were treated with MeJA. Seedlings insensitive to MeJA were identified and transplanted. The *jai1-1* mutant contains a 6.2-kb deletion in the *Coi1* gene (Li et al. 2004). The genotype of these plants was further confirmed by the presence of the deletion in the *Coi1* gene, using multiplex polymerase chain reaction (PCR) as described by Li and associates (2004) (data not shown).

Initially, tomato plants were screened in "choice" assays in which insects were allowed to choose between the wild-type cv. CM and *jai1-1* mutant plants. Choice assays therefore allowed the determination of plant-specific aphid feeding preferences. Two weeks after aphid exposure, the number of aphids on the *jai1-1* mutant was significantly higher ($P < 0.05$) than the number of aphids on the wild-type parent (Fig. 1).

To further evaluate the role of JA in aphid resistance, "no-choice" aphid assays were used, in which aphids were confined to a single leaflet. One-day-old adult aphids were used to accurately evaluate aphid survival and avoid variation resulting from age-dependent insect responses. The survival and fecundity of aphids were monitored on a daily basis on the *jai1-1* mutant and the wild-type parent. Except for day 16, the percent daily aphid survival was not significantly different on *jai1-1* mutant plants compared with cv. CM plants ($P > 0.05$) (Fig. 2A). On day 16, a significant difference ($P < 0.01$) in aphid survival was noted because one aphid survived on the *jai1-1* mutant, while all aphids were dead on the wild-type parent (Fig. 2A). Potato aphids have a viviparous mode of reproduction, and progeny were counted on a daily basis to evaluate aphid fecundity. The average number of progeny per day per aphid on the *jai1-1* mutant was not significantly different from the number of progeny on cv. CM ($P > 0.001$) (Fig. 2B). Similarly, the total number of progeny per aphid was not significantly different ($P > 0.05$) on *jai1-1* mutant compared with cv. CM (Fig. 2C).

Genetic cross and identification of homozygous *Mi-1 jai1* plants.

The tomato *jai1-1* mutant does not contain the *Mi-1* gene. To evaluate whether *Coi1*-dependent signaling plays a role in *Mi-1*–mediated aphid resistance, we introduced *Mi-1* in the *jai1-1* mutant background. Genetic crosses were performed between VFN tomato and the *jai1-1* mutant, F1 plants were allowed to self, and the F2 population was evaluated for sensitivity to MeJA and the presence of a deletion in the *Coi1* gene. F2

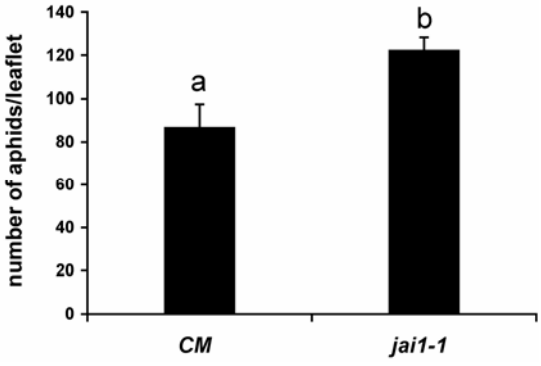

Fig. 1. A choice assay on tomato *jai1-1* mutant and wild-type parent cv. Castlemart (CM) plants. Six-week-old tomato plants were exposed to potato aphids in an insect cage in a greenhouse. Plants were evaluated 14 days after exposure to aphids. Ten plants per genotype were used. The experiment was performed twice with similar results. Data representing means from one experiment are presented. Error bars indicate ± standard error. Bars with different letters denote a significant difference at $P < 0.05$.

plants insensitive to MeJA were further evaluated for the presence of the *Mi-1* gene, using the linked marker REX-1 (data not shown) (Williamson et al. 1994). REX-1 is a codominant marker that allows for the identification of the *S. peruvianum* introgressed region spanning *Mi-1*. Plants homozygous for the *Mi-1* locus and for the *coi1* deletion were used for further evaluations.

Expression of JA regulated wound-induced genes in *Mi-1 jai1* plants

To assess the integrity of the JA signaling pathway in the homozygous *Mi-1 jai1* plants (referred to as VFN × *jai1-1*), the expression of *allene oxide synthase 1* (*AOS1*), an octadecanoid pathway enzyme encoding a cytochrome P450, and *Pin1* were evaluated (Howe et al. 2000). *AOS1* and *Pin1* are induced after wounding early and late, respectively (Ryan 2000). Tomato leaflets either wounded or exposed to potato aphids were used in these experiments. In VFN leaflets, *AOS1* transcripts accumulated to high levels at 4 h after wounding and were absent at 24 h (Fig. 3). Similarly, *AOS1* transcripts were expressed transiently at 4 h but to a lesser extent in both *jai1-1* mutant and VFN × *jai1-1* leaflets (Fig. 3). In contrast, *AOS1* transcripts were not detected at 6 and 12 h after aphid feeding on any of the three genotypes tested (Fig. 3). In VFN leaflets, *Pin1* transcripts accumulated to very high levels at both 4 and 24 h after wounding (Fig. 3). *Pin1* transcripts accumulated in VFN leaflets 6 and 12 h after aphid feeding, confirming an earlier report (Martinez de Ilarduya et al. 2003). In contrast, no *Pin1* transcripts were detected in either *jai1-1* or VFN × *jai1-1* leaflets after wounding or aphid feeding, indicating that the JA signaling pathway is compromised in these plants (Fig. 3).

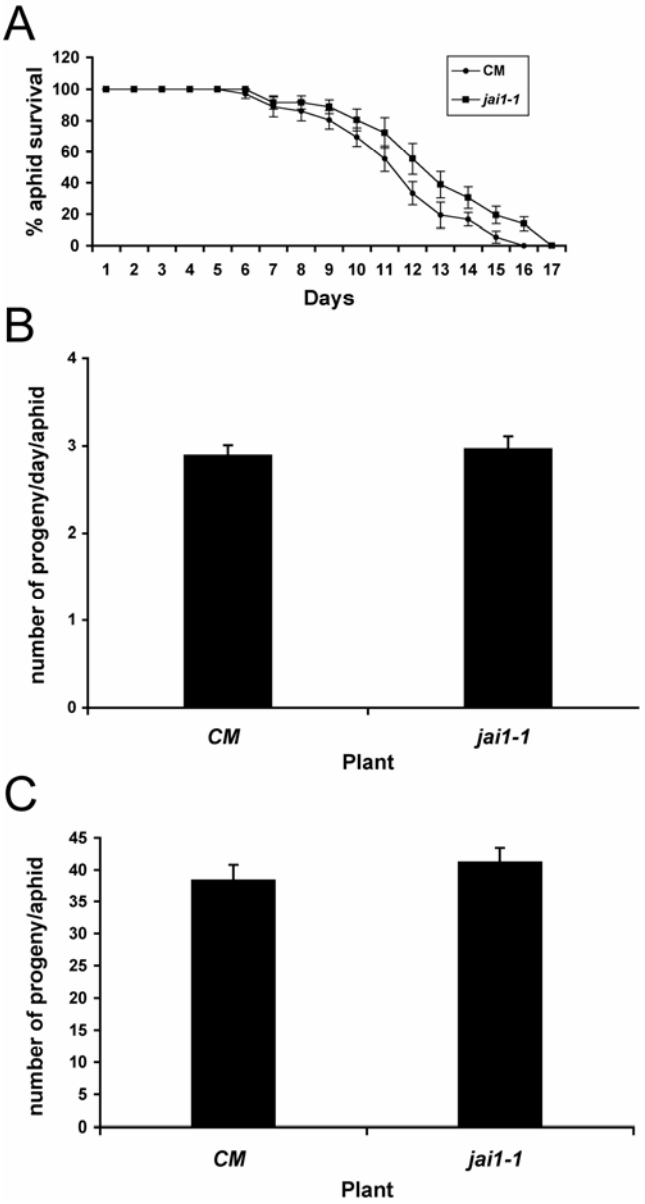

Fig. 2. Aphid survival and fecundity on tomato wild-type cv. Castlemart (CM) and *jai1-1* mutant plants. Four-week-old tomato plants were used in no-choice assays. A single leaflet per plant was infested with four one-day-old aphids using clip cages, and aphid survival and fecundity were monitored daily. Nine plants of each genotype were used. Error bars indicate ± standard error. The experiment was performed twice with similar results. Data representing one experiment are presented. **A,** Daily aphid survival. Statistical analysis was performed on arcsin-transformed data, and significant differences at $P < 0.05$ are indicated by an (*). **B,** Daily aphid reproduction. **C,** Aphid reproduction. Bars with similar letters denote no significant difference at $P > 0.001$.

Potato aphid survival on *Mi-1 jai1* plants.

Both choice and no-choice assays with potato aphids were used to evaluate *Mi-1* plants compromised in the JA signaling pathway. In choice assays, in which insects were allowed to choose among *jai1-1*, VFN, or VFN × *jai1-1* plants, significantly higher numbers of aphids were present on *jai1-1* plants compared with VFN ($P < 0.001$) and VFN × *jai1-1* plants ($P < 0.001$) (Fig. 4A and B). There was no significant difference between number of aphids on VFN × *jai1-1* compared with VFN plants ($P > 0.001$), indicating that the *Coi1*-dependent signaling pathway is not required for *Mi-1*–mediated aphid resistance (Fig. 4A and 4B). To further confirm these results, a no-choice assay was performed with one-day-old adult aphids. The no-choice assay confirmed the results obtained in the choice assay. The number of aphids on VFN × *jai1-1* plants was similar to the number of aphids on VFN plants ($P > 0.001$) but was significantly higher than the number of aphids on *jai1-1* mutant plants ($P < 0.001$) (Fig. 4C). Similarly, there was a significantly lower number of aphids on VFN compared with *jai1-1* plants ($P < 0.001$) (Fig. 4C).

DISCUSSION

Coi1 does not play a role in *Mi-1*–mediated aphid resistance.

Our results do not support a role for a *Coi1*-dependent signaling pathway in *Mi-1*–mediated resistance to potato aphids. In spite of this finding, transcripts of both defense-related proteins *Pin1* (Fig. 3) and *Pin2* (Martinez de Ilarduya et al. 2003) are induced early after aphid feeding on resistant plants, suggesting that tomato JA-dependent defenses are activated in the incompatible interaction. In general, faster and higher levels of transcript accumulation is associated with incompatible interactions (Tao et al. 2003). Since temporal expression of *Pin1* and *Pin2* was similar in both compatible and incompatible interactions, JA-dependent plant defense could contribute to basal defense rather than to *Mi-1*–mediated defense (Martinez de Ilarduya et al. 2003).

Mi-1–mediated aphid and nematode resistance requires the SA signaling pathway (Branch et al. 2004; Li et al. 2006). Introduction of *NahG* in the *Mi-1* background abolished aphid resistance in leaves, allowing aphids to multiply on *Mi-1* plants (Li et al. 2006). Application of benzothiadiazole, a SA analog, was able to rescue the susceptible phenotype in *Mi-1 NahG* plants, indicating that SA is required for *Mi-1*–mediated aphid defense (Li et al. 2006). In this article, it was demonstrated that

Mi-1–mediated aphid resistance is unaffected by the absence of a functional JA pathway, indicating that JA is not required for resistance. The requirement of SA but not JA in *Mi-1*–mediated aphid defense in tomato demonstrates that gene-for-gene resistance against aphids functions through similar defense signaling pathways as to most plant pathogens (Kaloshian 2004; Kaloshian and Walling 2005).

Gene regulation in tomato with the *jai1* mutation.

AOS genes are induced transiently after wounding (Howe et al. 2000; Sivasankar et al. 2000) and application of MeJA (Li et al. 2004; Sivasankar et al. 2000). The lack of observed *AOS1* expression after aphid feeding on tomato genotypes is likely due to timing of tissue collection or the magnitude of the response. In a previous study, *AOS1* transcripts accumulated locally to high levels 1 and 2 h after wounding and decreased to nondetectable levels by 8 h (Lee and Howe 2003). Our aphid infestation timecourse (6 and 12 h after infestation) was selected to allow for the detection of *Pin1* transcripts (Martinez de Ilarduya et al. 2003). Tissue collection at earlier timepoints might have allowed detection of *AOS1* transcripts. Alternatively, the accumulation of *AOS1* transcripts by aphid feeding is lower than our detection limit.

A decrease in *AOS1* transcript levels was observed after wounding in *jai1-1* and VFN × *jai1-1* plants compared with VFN, reflecting a similar pattern as that shown for *AOS2* in response to exogenous MeJA treatment of *jai1-1* compared with wild-type plants (Li et al. 2004) and indicating that wound-inducible *AOS1* transcript accumulation is also partly *Coi1* dependent.

The lack of *Pin1* transcript accumulation after wounding in *jai1-1* and VFN × *jai1-1* plants confirmed earlier findings that expression of wound-induced proteinase inhibitors is *Coi1* dependent (Li et al. 2001). Similarly, the lack of *Pin1* transcript accumulation in aphid-infested leaf tissue indicated that the aphid-induced *Pin1* expression is also regulated by *Coi1* (Fig. 3). *Pin1* transcript accumulation is most likely due to wounding caused by aphid feeding. Although aphid stylets penetrate host tissue mainly intercellularly to reach the sieve element where they feed, intracellular penetration also occurs. In fact, 27% of the probes by potato aphids penetrate tomato tissue intracellularly, which may explain the induction of wound responses (Kaloshian et al. 2000). However, the JA-dependent wound signaling pathway did not contribute to aphid defense, since *jai1-1* mutant and wild-type tomato plants supported similar levels of aphid fecundity and survival.

The role of JA-regulated signaling in basal defense to aphids.

A differential role for JA is indicated depending on the aphid assay used. A small but significantly different increase in colonization of aphids was observed on the *jai1-1* mutant as compared with the wild-type parent in the choice assay (Fig. 1). Since no significant differences in aphid survival and fecundity was observed between *jai1-1* mutant and wild-type plants, the larger number of aphids observed on *jai1-1* plants could be due to changes in the oxylipin signature of this mutant that could enhance aphid attraction to *jai1-1* plants.

Eliminating insect choice between genotypes and movement between plants resulted in no difference in aphid survival or fecundity on *jai1-1* plants as compared with wild type, indicating no role for *Coi1*-dependent basal plant defense against potato aphids. In several plant species, stronger activation of SA- than JA-regulated genes by aphid feeding have been observed (Martinez de Ilarduya et al. 2003; Moran and Thompson 2001; Zhu-Salzman et al. 2004). Since cross talk exists between SA and JA signaling pathways, this and other information have lead to speculations that aphids might manipulate plant defenses by activating ineffective SA responses to suppress effective JA responses (Thompson and Goggin 2006; Zhu-Salzman et al. 2004). Our results indicate that tomato–potato aphid interaction does not follow this model. Although aphids activate both SA and JA responses, eliminating the JA response did not alter aphid defense.

In *Arabidopsis*, the role of the JA-regulated pathway in aphid defense is not well defined. For example, the *Arabidopsis coi1* mutation had no effect on *Myzus persicae* population growth (Mewis et al. 2005). In contrast, the same researchers reported more rapid growth of the specialist aphid *Brevicoryne brassicae* on the *coi1* mutant compared with the wild-type parent (Mewis et al. 2005). In addition, the *Arabidopsis cev1* mutant, which expresses JA-dependent responses constitutively, supported low *Myzus persicae* population growth, supporting a role for the JA-regulated defense in *Arabidopsis* to aphids (Ellis et al. 2002). Since the *cev1* mutation has pleiotropic effects, it is difficult to reach conclusions using this mutant (Ellis and Turner 2001). These results indicate that, in *Arabidopsis*, the role of the JA-regulated pathway in basal resistance to aphids remains controversial and suggests that different plant species may involve distinct defense signaling pathways (Kaloshian and Walling 2005; Thompson and Goggin 2006).

Exogenous application of MeJA reduced greenbug aphid *(Schizaphis graminum*) attraction to sorghum seedlings (Zhu-

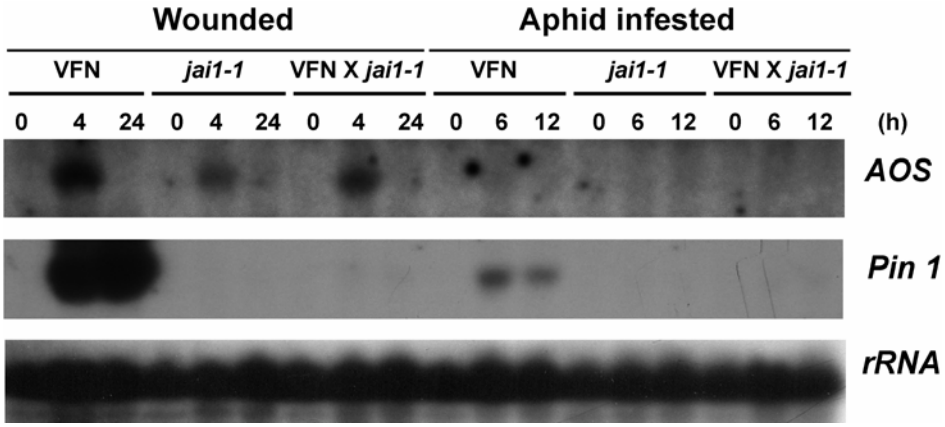

Fig. 3. Transcript accumulation in leaflets of tomato cv. VFN (*Mi-1/Mi-1 Jai1/Jai1*), *jai1-1 (mi/mi jai1/jai1)*, and VFN × *jai1-1* (*Mi-1/Mi-1 jai1/jai1*) after wounding or potato aphid infestations. Leaflets of seven-week-old tomato plants were either wounded or infested with potato aphids. Wound samples were collected at 0, 4, and 24 h after wounding. Aphid-infested samples were collected 0, 6, and 12 h after infestations. Total RNA was isolated, and the RNA blot was hybridized to probes for *LeAOS1* (*AOS)*, proteinase inhibitor I (*Pin1)*, and 18S rRNA as a loading control.

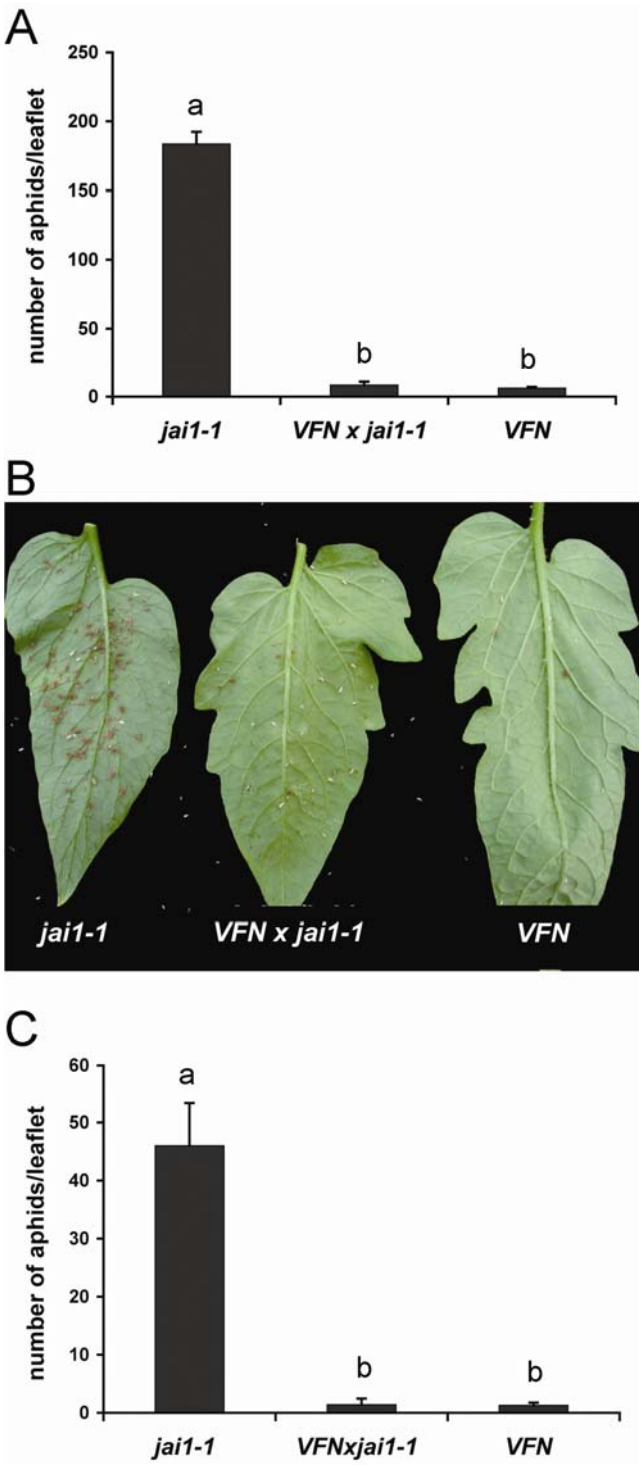

Fig. 4. Aphid assays of tomato cv. VFN (*Mi-1/Mi-1 Jai1/Jai1*), *jai1-1 (mi/mi jai1/jai1)*, and VFN × *jai1-1* (*Mi-1/Mi-1 jai1/jai1*) plants. **A,** Aphid choice assay with seven-week-old tomato plants in an insect cage in a greenhouse. Plants were evaluated 14 days after exposure to aphids. Eight plants per genotype were used. The experiment was performed twice with similar results. Data representing means from one experiment are presented. Error bars indicate ± standard error. Bars with different letters denote a significant difference at $P < 0.001$. **B,** Phenotype of tomato leaflets infested with potato aphids in the choice assays. **C,** Aphid survival in a no-choice assay. Individual leaflets of seven-week-old tomato were infested with four one-day-old aphids using leaf cages. Four leaflets per plant and eight plants of each genotype were infested. The number of aphids was evaluated 14 days after aphid infestation. The experiment was performed one time. Error bars indicate ± standard error. Bars with different letters denote a significant difference at $P < 0.001$.

Salzman et al. 2004) and *Myzus persicae* growth on *Arabidopsis* (Ellis et al. 2002). Similarly, exogenous JA application reduced potato aphid host preference, survival, and fecundity on susceptible tomato plants lacking *Mi-1* (Cooper and Goggin 2005). However, this negative effect was not seen on resistant *Mi-1* tomato plants (Cooper and Goggin 2005). These results suggest that artificial augmentation of the JA pathway enhances aphid basal resistance and this effect is not exhibited in resistant plants. Alternatively, the exogenous application of JA might affect the pattern of other oxylipins that might be involved in aphid resistance (Avdiushko et al. 1995; Kohlmann et al. 1999). Recently, a role for the JA precursor 12-oxo-phytodienoic acid independent of JA was described (Stintzi et al. 2001; Taki et al. 2005). It is, therefore, possible that a *Coi1*-independent signaling pathway mediated by other oxilipins might play a role in aphid defense.

MATERIALS AND METHODS

Plant materials and growth conditions.

The tomato cultivars and mutants used in this study were: VFN (*Mi-1/Mi-1*), UC82B (*mi/mi*), CM (*mi/mi*), and *jai1-1* mutant in CM background (Li et al. 2004). Seeds were sown in seedling trays filled with organic planting mix (Sun Gro Horticulture, Bellevue, WA, U.S.A.) or were sown and grown directly in Jiffy peat pots (Jiffy Product of America, Inc., Batavia, IL, U.S.A.). To promote uniform germination, seedling trays were maintained in an enclosed greenhouse structure with misters. After germination, seedlings were grown in a pesticide-free greenhouse inside large plant cages to avoid insect infestations, with temperatures ranging from 22 to 26°C. Two to three weeks after germination, seedlings were transplanted into plastic pots (10 cm in diameter, 17 cm deep) filled with a 1:1 proportion of University of California mix II and sand. All seedlings were supplemented with Osmocote (17-6-10) (Sierra Chemical Company, Milpitas, CA, U.S.A.) and were fertilized biweekly with Tomato MiracleGro (18-18-21) (Stern's MiracleGro Products, Port Washington, NY, U.S.A.).

Aphid rearing.

A colony of a parthenogenetic *Mi-1*–avirulent potato aphid, *Macrosiphum euphorbiae*, was maintained on susceptible tomato cv. UC82B plants inside an insect cage in a pesticide-free greenhouse at 22 to 26°C. Artificial lighting was supplemented during the winter season to increase day length to a 16 h photoperiod.

To obtain one-day-old adult aphids, adult apterous (wingless) aphids were transferred to tomato seedlings and were allowed to lay progeny. Seedlings were maintained in an insect cage in a pesticide-free greenhouse. About 24 h after aphid infestation, the adult aphids were removed, using a Pasteur pipette hooked to an aspirator, and the first instars were allowed to develop to maturity.

Chemical screen of seedlings with MeJA.

Seedlings were screened for sensitivity to MeJA according to Li and associates (2004). Briefly, surface-sterilized tomato seeds were germinated on filter paper until the roots were 2 cm in length. The germinated seedlings were exposed to MeJA by saturating the filter paper with 1 mM MeJA. Approximately 1 day later, MeJA insensitive seedlings were selected and transplanted.

Genetic crosses.

Genetic crosses were performed between VFN and *jai1-1* mutant. Since mutation in the *Coi1* gene in tomato results in sterility due to maternally-controlled improper seed maturation

(Li et al. 2004), we used VFN as the female parent and the *jai1-1* mutant as the source of pollen. To obtain homozygous *jai1-1* pollen, seedlings segregating for the locus were evaluated for MeJA insensitivity and the presence of the *coi1* deletion as described above.

Molecular genotyping.

Rex-1 and *Coi1* loci were amplified using PCR. The Rex-1 primers and amplification conditions are described by Williamson and associates (1994), and the tomato *Coi1* primers and amplification conditions are described by Li and associates (2004).

Timecourse aphid infestations.

For timecourse transcript analyses, 25 apterous potato aphid adults and nymphs were caged onto individual leaflets from the fourth or fifth leaves of 7-week-old tomato plants. The leaf cages used in this experiment were as described by Li and associates (2006). Three cages were used per plant, and two plants were used for each timepoint/genotype combination. To collect leaf tissue, cages were removed and leaflets were sprayed with 1% sodium dodecyl sulfate (SDS) to force aphids to withdraw their stylets. The aphids were then carefully removed with a paintbrush. Tomato leaflets were excised using a razor blade, were immediately frozen in liquid nitrogen, and were stored at –80°C.

Aphid choice bioassay.

In choice assays, test plants were moved into large cages inside a pesticide-free greenhouse. Several pots of aphid-infested, four-week-old susceptible tomato seedlings, 20 seedlings per pot (12 cm diameter, 10 cm deep), were distributed within a cage. Since the aphid source plants were smaller in size compared with the screened plants, pots containing the aphid source were raised to the mid level of the screened plants to allow easy access of aphids to the experimental plants. At 14 days after aphid exposure, aphids on the most-infested three leaflets of each plant were counted. The experiment was performed twice.

Aphid no-choice bioassay.

Two distinct no-choice aphid bioassays were performed. In the first no-choice assay, four one-day-old adult apterous aphids were caged onto the abaxial leaf surface of a single leaflet of a four-week-old tomato using a clip cage. Nine replicates of each genotype were used. Plants were placed randomly on a bench in a pesticide-free greenhouse maintained at 22 to 26°C. Aphid survival was monitored and recorded on a daily basis. To evaluate aphid fecundity, first instar progeny were counted and removed, using an aspirator on a daily basis. The experiment was performed twice.

In the second no-choice bioassay in which *Mi-1* tomato plants were involved, four one-day-old adult apterous aphids were caged onto individual leaflets of seven-week-old tomato, as described for the timecourse infestation. Plants were placed randomly on a bench in a pesticide-free greenhouse maintained at 22 to 26°C. Plants were evaluated by counting the number of aphids on each caged leaflet 14 days after aphid infestations.

Statistical analyses.

Data having three or more treatments were analyzed using analysis of variance (ANOVA) on raw data using the statistical package SAS (v9.1 for Windows, SAS Institute, Inc., Cary, NC, U.S.A.). Pairwise comparisons were performed using Fisher's probable least-squares difference. Differences in means for two genotypes were performed, using a two-sample *t* test also using the statistical package SAS. Data in percentage values were arcsin-transformed before analysis to correct for normality and differences in variance between treatments. Daily aphid survival across days was analyzed using repeated measures ANOVA to account for the correlations over time in the consecutive data.

RNA isolation and gel blot analysis.

Total RNA was isolated as described previously (Martinez de Ilarduya et al. 2001). Total RNA (15 μg per lane) was separated on a 1.2% agarose/formaldehyde gel and was transferred to a nylon membrane (Osmonics, Inc., Minnetonka, MN, U.S.A.) in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). The gel was stained with ethidium bromide to assure equal loading per lane. A *Pin1* cDNA clone was kindly provided by C. A. Ryan (Washington State University, Pullman, WA, U.S.A.). The tomato expressed sequence tag clone cLEC9C14 was used as probe for *AOS1*. A soybean rRNA clone was used as a control of equal transfer between lanes. DNA probes were labeled with ^{32}P-α-dCTP, using the Redi-prime labeling kit (Amersham, Arlington Heights, IL, U.S.A.) according to the manufacturer's instructions. RNA blots were prehybridized for 2 h at 42°C and were hybridized for 16 h at 42°C, as described by Martinez de Ilarduya and associates (2001). The final blot wash was in 0.5× SSC, 0.1% SDS at 65°C for 30 min. Hybridization results were visualized by autoradiography using Kodak XAR-5 film. Probes were stripped by pouring boiling 0.5% SDS on the membrane. Before reuse, the membrane was checked for complete removal of probe.

Wound treatment.

Tomato leaflets were wounded by crushing with a pair of pliers on two locations on the midrib. Three leaves per plant were wounded, two plants were used for each timepoint, and tissue was pooled. Unwounded leaflets from the two plants were sampled at the beginning of the experiment. Wounded leaflets were harvested at 4 and 12 h after wounding. Sampled leaf tissue was immediately frozen in liquid nitrogen and stored at –80°C.

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