MPMI Vol. 16, No. 8, 2003, pp. 699–708. Publication no. M-2003-0529-01R.

Aphid-Induced Defense Responses in *Mi-1*-Mediated Compatible and Incompatible Tomato Interactions

Oscar Martinez de Ilarduya, QiGuang Xie, and Isgouhi Kaloshian

Department of Nematology, 1303 Webber Hall, University of California, Riverside, CA 92521 U.S.A.

Submitted 11 April 2003. Accepted 19 April 2003.

The tomato *Mi-1* gene confers resistance to three species of root-knot nematode and potato aphid. We studied changes in expression of jasmonic acid (JA)- and salicylic acid (SA)-dependent defense genes in response to potato and green peach aphids. We determined changes in three PR proteins, lipoxygenase and proteinase inhibitors I and II transcripts, locally and systemically in both compatible and incompatible interactions in tomato. Transcripts for *PR-1* were detected earlier and accumulated to higher levels in the incompatible than in the compatible potato aphid/tomato interactions. The transcript profiles of the other genes were similar in compatible compared with incompatible interactions. *Pin1* and *Pin2* RNAs were detected early and transiently in both compatible and incompatible interactions. In tomato plants containing *Mi-1,* systemic expression of *PR-1* and *GluB* was detected in both compatible and incompatible interactions at 48 h after infestations with either aphid. These results suggest that aphid feeding involves both SA and JA/ethylene plant defense signaling pathways and that *Mi-1*-mediated resistance might involve a SA-dependent signaling pathway. Potato aphid feeding generated reactive oxygen species in both compatible and incompatible interactions. However, a hypersensitive response was absent in the *Mi-1*-mediated resistance response to potato aphids. Reciprocal grafting experiments revealed that resistance is cell autonomous, and local expression of *Mi-1* is required for *Mi-1*-mediated resistance against the potato aphid.

The potato aphid, *Macrosiphum euphorbiae* Thomas, is a serious pest of tomato worldwide and often results in significant yield losses. Feeding by this insect causes leaf chlorosis, stunting of shoots, and dieback. Besides the direct feeding damage, aphids also can cause indirect damage by transmitting plant viruses and supporting sooty mold growth. Resistance to potato aphid was identified in tomato plants containing the *Mi-1* gene (Kaloshian et al. 1995). Initially, the *Mi-1* gene was introgressed into tomato, *Lycopersicon esculentum* Mill, from its wild relative *L. peruvianum* as a source of resistance to root-knot nematodes, *Meloidogyne* spp. (Smith 1944). After *Mi-1* was cloned, it was shown that *Mi-1* confers resistance to nematodes and the potato aphid (Milligan et al. 1998; Rossi et al. 1998). Two transcribed genes, with over 92% identity, are present in the *Mi-1* locus. Only one of these genes confers resistance to both nematodes and potato aphid. *Mi-1* confers resistance to three species of root-knot nematodes: *M. arenaria*, *M. incognita*, and *M. javanica*. Both nematode resistance and aphid resistance seem to behave according to the gene-for-gene model, as field isolates of root-knot nematodes and potato aphids that overcome resistance mediated by *Mi-1* have been identified (Goggin et al. 2001; Kaloshian et al. 1996; Rossi et al. 1998).

Although resistance to root-knot nematodes and potato aphid is conferred by the same gene, the resistance to these organisms is regulated differently. Tomato plants containing *Mi-1* are resistant to nematodes early in root development. In contrast, the *Mi-1*-mediated resistance against potato aphid is developmentally regulated, with fully expanded leaves becoming resistant only when plants are four to five weeks of age (Kaloshian et al. 1995). In these plants, fully expanded leaves are resistant to aphids irrespective of leaf position on the plant (Kaloshian et al. 1997). However, expanding leaves remain aphid-susceptible throughout the life of the plant (I. Kaloshian, *unpublished data*). It is not clear what controls the developmental regulation of the resistance to aphids. Most likely, *Mi-1* is not involved, since *Mi-1* transcripts are present in roots and leaves very early in development and, therefore, are not correlated with aphid resistance. Furthermore, *Mi-1* RNA levels do not change after exposure to either root-knot nematodes or potato aphids (Martinez de Ilarduya and Kaloshian 2001).

Mi-1 belongs to the family of resistance genes with a leucine zipper, a nucleotide-binding site, and leucine-rich repeats and is the only example of a gene conferring resistance to two distinct animals (Milligan et al. 1998). To date, dual specificity has been reported for the *Arabidopsis thaliana* gene *RPM1*and the tomato gene *Pto* (Grant et al. 1995; Kim et al. 2002). Both RPM1 and Pto recognize two nonhomologous avirulence gene products present in the same bacterium. It is likely that Mi-1 also recognizes two distinct avirulence products, one from the nematode and the other from the potato aphid. Little is known about how *Mi-1* mediates resistance to nematodes and aphids. The resistance mediated by *Mi-1* to root knot nematodes is accompanied by a hypersensitive response (HR) in root tissue near the head of the feeding infective stage juvenile. In response to HR-induced cell death, nematodes stop feeding and either die from starvation or most likely exit the root.

In the case of potato aphid, it is not clear whether HR is involved in the incompatible tomato interaction. No necrotic spots are seen on tomato leaflets infested with potato aphids, and the nature of the resistance mechanism is not well understood. It is apparent that the *Mi-1*–mediated resistance has profound effects on the development, longevity, and fecundity of the potato aphid (Kaloshian et al. 1997). Adult aphids and

Corresponding author: I. Kaloshian; E-mail: isgouhi.kaloshian@ucr.edu; Telephone: 1-909-787-3913; Fax: 1-909-787-3719.

Current address of O. Martinez de Ilarduya: Centro de Investigaciones Agrarias de Mabegondo, Apartado 10, 15080 A Coruña, Spain.

nymphs die as early as 24 h after exposure to resistant plants. Death likely occurs due to starvation and desiccation, since aphids appear to recover fully when transferred from resistant to susceptible tomato lines (Kaloshian et al. 1997). Electronic monitoring studies of aphid feeding behavior indicate that aphids are able to penetrate *Mi-1*–containing tomato leaves and successfully reach sieve elements with their stylets. However, they ingest only limited amounts of vascular fluids from resistant tomato plants compared with that from susceptible plants (Kaloshian et al. 2000).

Limited information is available about changes in gene expression in plants in response to incompatible aphid-plant interaction and the mechanism of resistance. The existing information addresses changes in protein levels of pathogenesis-related (PR) proteins. Increases in levels of chitinases and β-1,3-glucanases have been reported in resistant but not susceptible barley upon infestation with the birdcherry-oat aphid *Rhopalosiphum padi* L. and in both resistant and susceptible sorghum exposed to the greenbug *Schizaphis graminum* Rondani (Forslund et al. 2000; Krishnaveni et al. 1999).

A number of resistance genes trigger a well-defined set of defense related genes, including PR proteins (Glazebrook 2001). Defense responses are usually dependent on either the salicylic acid (SA) pathway or jasmonic acid (JA) and ethylene-dependent pathways (Glazebrook 2001; Penninckx et al. 1998; Thomma et al. 2000). In addition, a certain level of cross talk between these pathways with both synergistic and inhibitory effects has been shown (Thomma et al. 2001). Over the past decade, similarities have emerged between defense responses to phloem-feeding insects and plant pathogens (Ellis et al. 2002; Moran and Thompson 2001; Walling 2000). To investigate the mechanism of *Mi-1*–mediated resistance to potato aphids and to determine which sets of defense response genes were induced in the *Mi-1*–mediated response to potato aphid, we assessed the expression profiles of a number of these genes.

We determined changes in three PR proteins, lipoxygenase and proteinase inhibitors I and II gene transcripts, in both compatible and incompatible potato aphid-tomato interactions. For comparison, *Mi-1* and *mi-1* plant responses to a second aphid species, the green peach aphid *Myzus persicae* Sulzer were monitored. No resistance is present in tomato against the green peach aphid. In addition, to understand the mechanism of resistance mediated by *Mi-1* against the potato aphid, we investigated the production of reactive oxygen species (ROS) and the correlation with the presence or absence of HR in the incompatible interaction. We also investigated whether a translocating signal that could pass through a graft junction is involved in the *Mi-1*–mediated resistance to the potato aphid. Our results indicate that *PR-1* transcripts accumulate earlier in the *Mi-1*–mediated incompatible interaction than the compatible interactions. In addition, transcripts of genes involved in plant defense against pathogens and chewing insects are activated by aphid feeding in both compatible and incompatible tomato-aphid interactions. Although potato aphid feeding on tomato induces an oxidative burst, no HR is detected in the *Mi-1*–mediated resistance to potato aphids. Furthermore, the resistance mediated by *Mi-1* is not graft transmissable.

RESULTS

PR protein RNAs.

To determine whether there was a correlation between gene expression in compatible and incompatible tomato plant responses and resistance to aphids, we investigated the expression of a number of defense-response genes that are induced in gene-for-gene interactions. Previous work using electronic monitoring of aphid feeding behavior has shown that potato aphid feeding behavior on *Mi-1* tomato is altered within the first 16 h of aphid infestation (Kaloshian et al. 2000). Within this period, aphids are able to reach the phloem element, but they feed significantly less on resistant than on susceptible tomato leaves. In addition, aphids start dying as early as 24 h after feeding on resistant tomato plants. Based on this information, we chose to sample potato aphid-infested leaves from *Mi-1* (incompatible interaction) and *mi-1* (compatible interaction) plants at intervals within the first 48 h of exposure.

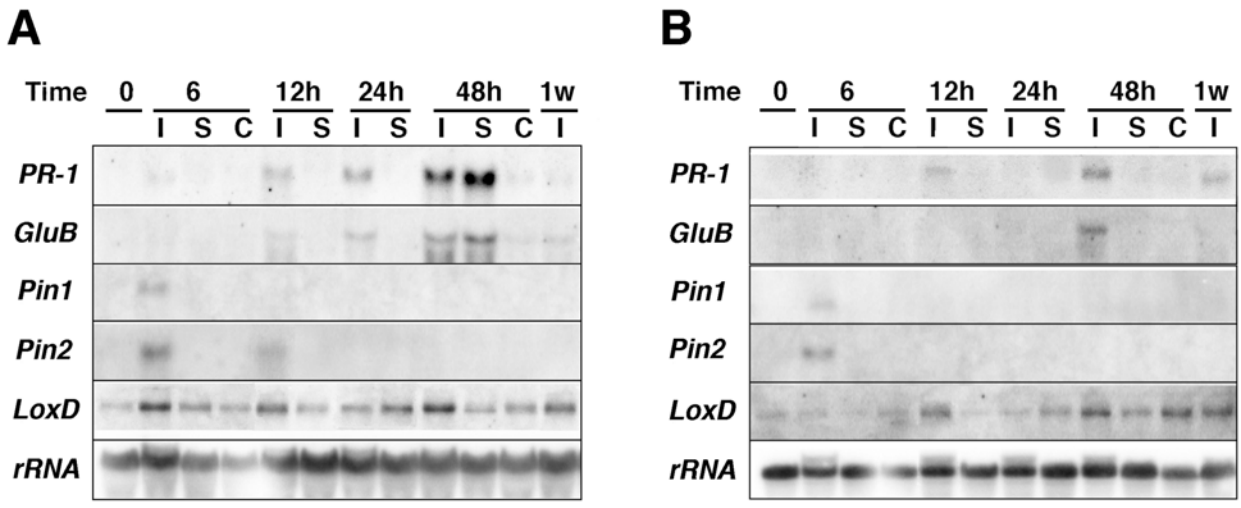

Fig. 1. Potato aphid infestation accumulated transcripts of defense-related genes in both compatible and incompatible tomato interactions. The near-isogenic cvs. Motelle (*Mi-1*) and Moneymaker (*mi-1*) were infested with potato aphids. The basal leaflet of the fourth fully expanded leaf of eight-week-old tomato plants was infested with an avirulent biotype of the potato aphid (40 at mixed stages). Samples of the infested leaflet (I) were collected at the time of infestation (0) and at 6, 12, 24, and 48 h and 1 week (1w) after aphid infestation. To assess whether transcripts accumulated systemically, the leaflet opposite the infested one (S) was collected 6, 12, 24, and 48 h after aphid infestation. Control samples (C) consisting of leaflets with empty cages were collected at every timepoint. The controls at the 12 and 24 h timepoints were run in duplicate blots and gave no hybridization signal but are not shown here. Total RNA (15 μg) from each sample was electrophoresed, was blotted onto nylon membrane, and was hybridized sequentially with ^{32}P-labeled defense gene probes. Hybridization with an 18S rRNA probe was used as a control to assess RNA loading and transfer. **A,** RNA blot from the resistant Motelle (*Mi-1*) leaflets infested with potato aphids. **B,** RNA blot from the susceptible Moneymaker (*mi-1*) leaflets infested with potato aphids. Data from one representative infestation experiment is displayed.

The accumulation of *PR-1* (also known as P4) RNAs was determined. *PR-1* encodes an extracellular protein with antifungal activity, but its mode of action is unknown (Niderman et al. 1995). *PR-1* RNAs accumulate in response to exogenous methyl jasmonate, ethylene, and salicylic acid (SA) signal molecules (Chao et al. 1999; van Kan et al. 1995). In the incompatible interaction of Motelle (*Mi-1*) plants infested with potato aphid, *PR-1* transcripts accumulated faster and to higher levels than in the compatible interaction of Moneymaker (*mi-1*) plants (Fig. 1A and B). *PR-1* transcripts could be detected in infested leaflets as early as 6 h after infestation (hai) (Fig. 1A). RNA levels increased at 12 and 24 hai and reached maximum levels ($P < 0.05$) by 48 hai (Fig. 2A). Very low levels of *PR-1* transcripts could be detected one week after potato aphid infestation (Figs. 1A and 2A). *PR-1* expression was also observed in adjacent noninfested leaflets at 48 hai (Fig. 1A). No *PR-1* transcripts were detected in samples from the 0 timepoint or from leaflets with empty cages (Fig. 1A; data not shown), indicating that *PR-1* RNA accumulation was specifically elicited by potato aphid infestation.

In the compatible interaction on Moneymaker (*mi-1*) plants infested with potato aphid, *PR-1* transcripts were detected in infested leaflets at 12 hai (Fig 1B). *PR-1* RNA levels were similar at 12 and 24 hai, while significant increase was seen at 48 hai ($P < 0.05$) (Fig. 2A). *PR-1* RNAs were detected at very low levels one week after potato aphid infestation (Figs. 1B

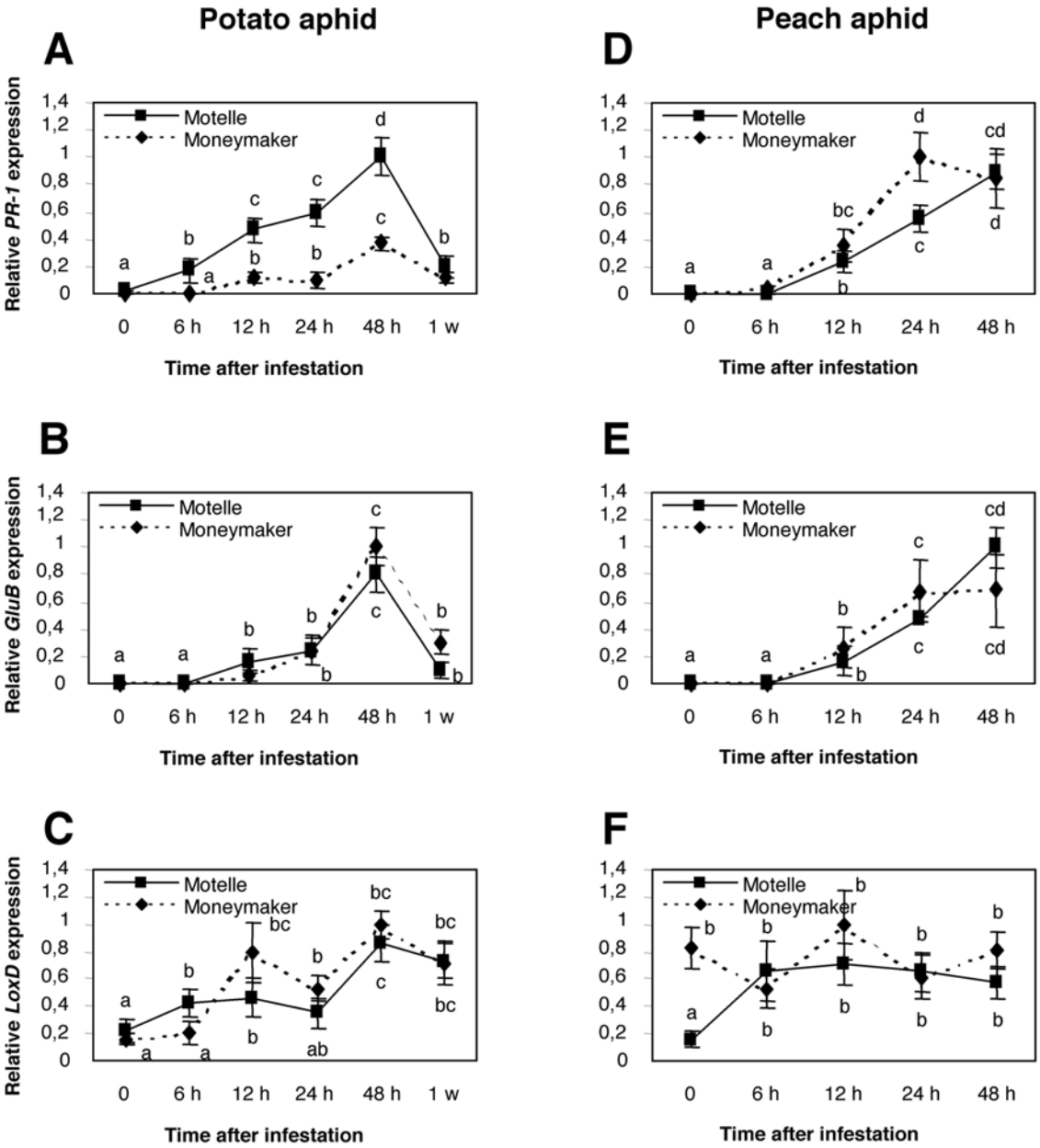

Fig. 2. Quantitation of defense gene RNAs in tomato cvs. Motelle (*Mi-1*) and Moneymaker (*mi-1*) after potato aphid and green peach aphid feeding. Green peach or potato aphids (40 at mixed stages) were caged on eight-week-old tomato leaflets. Samples were collected at the indicated timepoints after aphid infestation. RNAs were electrophoresed, blotted, and hybridized with ^{32}P-labeled *PR-1*, *GluB, LoxD*, and 18S rRNA probes. Signals were quantitated after normalization to rRNA levels in each lane. The maximum RNA level for each gene was given a value of 1.0, and the remaining samples were calculated as a proportion of this value. Error bars show standard deviation in two different blots from two independent experiments. **A,** *PR-1* transcript levels in tomato leaflets infested with potato aphids. **B,** *GluB* transcript levels in tomato leaflets infested with potato aphids. **C,** *LoxD* transcript levels in tomato leaflets infested with potato aphids. **D,** *PR-1* transcript levels in tomato leaflets infested with green peach aphids. **E,** *GluB* transcript levels in tomato leaflets infested with green peach aphids. **F,** *LoxD* transcript levels in tomato leaflets infested with green peach aphids. Error bars followed by the same letter within a graph are not statistically different at $P < 0.05$.

and 2A). No *PR-1* transcripts were detected in samples from the 0 timepoint or from leaflets with empty cages (Fig. 1B; data not shown). Similarly, no systemic accumulation of *PR-1* RNAs was detected in this compatible interaction (Fig. 1B).

Transcript levels of the intracellular basic β-1,3-glucanase (*GluB*), which accumulates in response to methyl jasmonate and ethylene signal molecules, were also investigated (Chao et al. 1999; van Kan et al. 1995). The temporal pattern and the level of *GluB* transcripts in local infested tomato leaflets were similar in both compatible and incompatible potato aphid interactions (Fig. 1A and B). *GluB* transcripts were observed in infested leaflets at 12 hai (Fig 1A). *GluB* RNA levels were similar at 12 and 24 hai and increased significantly by 48 hai ($P < 0.05$) (Fig. 2B). *GluB* RNAs were detected at very low levels one week after potato aphid infestation (Fig. 2B). *GluB* transcripts were undetectable in 0-h controls and in leaflets with empty cages (Fig. 1A; data not shown). Accumulation of *GluB* transcripts in adjacent noninfested leaflets was detected only in the incompatible Motelle *(Mi-1)* interaction at 48 hai (Fig. 1A).

For comparison, the changes in defense gene expression in response to an aggressive biotype of the green peach aphid, which causes visible feeding damage on tomato within a short period, was determined (I. Kaloshian, *unpublished data*). The interaction of the green peach aphid with Motelle (*Mi-1*) and Moneymaker (*mi-1*) plants is a compatible interaction. Motelle and Moneymaker leaves infested with green peach aphids had similar levels of *PR-1* transcripts by 12 hai ($P < 0.05$), followed by a steady increase of mRNA levels, reaching maximum levels at 24 hai in Moneymaker and at 48 hai in Motelle (Figs. 2D and 3A and B). In Motelle plants infested with green peach aphids, accumulation of *PR-1* transcripts in adjacent noninfested leaflets was detected at 48 hai (Fig. 3A). In contrast, in Moneymaker plants infested with the green peach aphids, also a compatible interaction, no accumulation of *PR-1* transcripts in adjacent noninfested leaflets was detected (Fig. 3B). Transcript levels and the temporal pattern of *GluB* were similar in local infested leaflets in the compatible interactions of green peach aphid on both Motelle and Moneymaker (Fig. 3A and B). *GluB* transcripts were detected 12 hai, with a steady increase in transcript levels until the last sampling timepoint at 48 hai ($P < 0.05$) (Fig. 2E). Accumulation of *GluB* transcripts in adjacent noninfested leaflets was detected only in the incompatible Motelle *(Mi-1)* interaction at 48 hai (Fig. 3A).

Expression of wound-response genes after aphid infestation.

Expression of the tomato proteinase inhibitors I (*Pin1*) and II (*Pin2*) genes were monitored to investigate the role of the methyl-JA–dependent pathway, which is induced by wounding and chewing insects (Graham et al. 1985a, 1985b). *Pin1* transcripts accumulated in potato aphid-infested leaflets only at 6 hai in both compatible and incompatible interactions (Fig. 1A and B). *Pin2* transcripts were detected at both 6 and 12 hai in the incompatible interaction and only at 6 hai in the compatible interaction (Fig. 1A and B). No accumulation of *Pin1* and *Pin2* transcripts in adjacent noninfested leaflets was observed (Fig. 1A and B). During the compatible interactions with the green peach aphid on both Motelle and Moneymaker tomato varieties, *Pin1* and *Pin2* transcripts were detected in the infested leaflets at both 6 and 12 hai, and RNA levels declined to nondetectable levels by 24 hai (Fig. 3A and B). There was some variation in the timing of *Pin1* and *Pin2* RNA accumulation in replicate experiments. For example, while these transcripts were not detected on the blot in Figure 3A at 6 hai, RNAs of both genes were detected at 6 hai in another experiment. *Pin1 and Pin2* transcripts were not detected in adjacent noninfested leaflets with green peach aphid feeding.

Accumulation of lipoxygenase, *LoxD,* transcripts was also investigated. Similar to *Pin1* and *Pin2*, *LoxD* RNAs accumulate in response to wounding, chewing insects, and JA (Heitz et al. 1997). A similar increase in *LoxD* transcript levels was detected in leaflets of Motellle and Moneymaker exposed to either potato aphids or green peach aphids at all timepoints tested (Figs. 1A and B, 2C and F, and 3A and B).

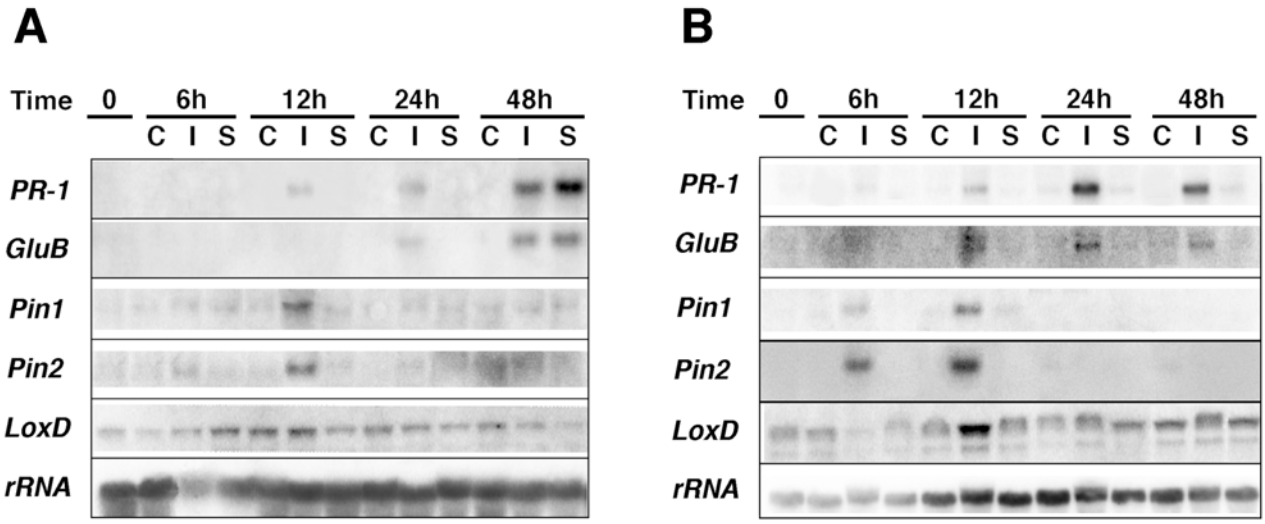

Fig. 3. Green peach aphid infestation accumulated transcripts of defense related genes in the compatible tomato interaction. The near-isogenic cvs. Motelle (*Mi-1*) and Moneymaker (*mi-1*) were infested with potato aphids. The basal leaflet of the fourth fully expanded leaf of eight-week-old tomato plants was infested with a virulent biotype of green peach aphid (40 at mixed stages). Samples from the infested leaflet (I) were collected at the time of infestation (0) and 6, 12, 24, and 48 h after aphid infestation. To assess systemic transcript accumulation, the leaflet opposite the infested one (S) was also collected at each timepoint. Similarly, control samples (C) consisting of leaflets with empty cages were collected at each timepoint. Total RNA (15 μg) from each sample was electrophoresed, was blotted onto nylon membrane, and was hybridized sequentially with ^{32}P-labeled defense gene probes. Hybridization with an 18S rRNA probe was used as a control to assess RNA loading and transfer. **A,** RNA blot from Motelle (*Mi-1*) leaflets infested with green peach aphids. **B,** RNA blot from Moneymaker (*mi-1*) leaflets infested with green peach aphids. Data from one representative infestation experiment is displayed.

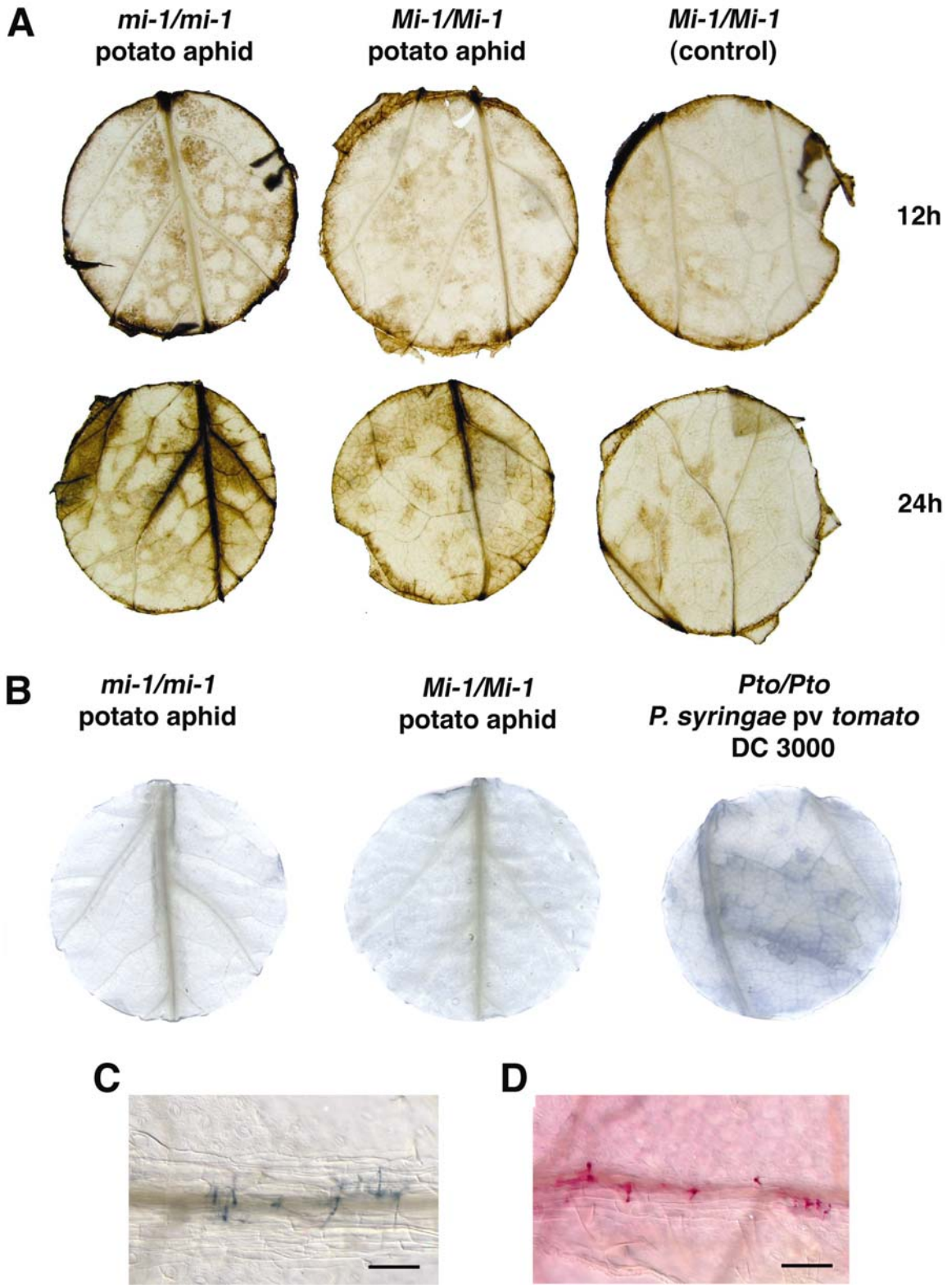

Fig. 4. Histochemical localization of H_2O_2 and cell viability in tomato leaves in response to potato aphid feeding. Eight-week-old tomato plants of cvs. Motelle (*Mi-1*) and Moneymaker (*mi-1*) were infested with potato aphids in clip cages. **A**, H_2O_2 localization in leaf tissue. Leaf disks from infested leaflets were processed 3, 6, 12, and 24 h after infestation. Disks were infiltrated and incubated with 3,3-diaminobenzidine for 6 h. Leaf disks from empty clip cages were used as negative controls. Results from the 12 and 24 h timepoints are shown. **B,** Tomato leaf tissue stained with trypan blue. Leaf disks from infested leaflets were stained with trypan blue 3, 6, 12, 24, and 48 h after infestation. Results from 48 h are shown. As a positive control for hypersensitive response, tomato leaves of cv. Rio Grande 76R were infiltrated with *Pseudomonas syringae* pv *tomato* DC3000 expressing *avrPto*. Dark blue stained cells are undergoing cell death. **C,** and **D**, Photomicrographs of tomato cv. Motelle 48 h after infestation with potato aphid. **C,** Potato aphid salivary sheaths stained with trypan blue and **D,** potato aphid salivary sheaths stained with acid fuchsin. Scale bar = 100 μm.

ROS accumulate upon potato aphid feeding, but HR is absent in the *Mi-1*–mediated aphid resistance.

The occurrence of oxidative burst in plants upon pathogen infection is well documented (Lamb and Dixon 1997). However, there is no information about such an event during feeding of piercing and sucking insects on plants. To determine whether the transcript accumulation of the plant defense genes was preceded by the production of ROS, we used a histochemical method to detect H_2O_2. The assay is based on the oxidation of diaminobenzidine (DAB), which turns brown in the presence of H_2O_2 (Orozco-Cardenas and Ryan 1999; Thordal-Christensen et al. 1997). The development of the DAB-H_2O_2 reaction product in tomato leaves in response to potato aphid feeding is shown in Figure 4A. H_2O_2 was detected at the wound site of mechanically wounded control leaflets (data not shown). No H_2O_2 was observed at 3, 6, or 12 hai with the potato aphid (Fig. 4A and data not shown). H_2O_2 was detected at 24 hai in primary and secondary veins of tomato leaflets in both compatible and incompatible interactions with the potato aphid (Fig. 4A).

Since ROS are correlated with HR and since *Mi-1*–mediated resistance to root-knot nematodes involves HR, we investigated whether HR was present in tomato leaves in the incompatible and compatible potato aphid interactions. Using a trypan blue staining protocol, no HR was detected in incompatible or compatible interactions at any timepoint tested (Fig 4B). However, trypan blue stained aphid salivary sheaths (Fig. 4C). The salivary sheath is a lipoproteinacous material that surrounds the stylets as they penetrate the plant (Miles 1999). The sheath is left behind after the aphid stops feeding and withdraws its stylets. We confirmed the nature of the stained material as the salivary sheath using acid fuchsin, which is known to stain insect salivary sheaths, including aphid sheaths (Fig. 4D) (Backus et al. 1988). As a positive control for HR, tomato leaflets of cv. Rio Grande 76R (*Pto/Pto*) were infiltrated with *Pseudomonaas syringae* pv. *tomato* DC3000 expressing *avrPto.* Dead cells in these leaflets were stained dark blue (Fig. 4B), indicating occurrence of the HR.

Transmission of aphid resistance in grafts.

To determine if the resistance mediated by *Mi-1* was systemically translocated through a graft, near-isogenic tomato cultivars Sun6082 (*Mi-1*) and CastlerockII (*mi-1*) were used in grafting experiments. Chimeric grafts were made between resistant (*Mi-1*) and susceptible (*mi-1*) tomato, using one as the rootstock and the other as the scion (Fig. 5A and B). Grafted controls were made by simply cutting and grafting onto the same variety. Three to four weeks after grafting, five grafted plants of each of the graft combinations (*Mi-1/mi-1*, *mi-1/Mi-1*, *Mi-1/Mi-1*, and *mi-1/mi-1*) were used in aphid infestation experiments. Potato aphid survival was monitored daily on leaves on either side of the graft unions. On chimeric grafts with *Mi-1* scions grafted onto *mi-1* rootstocks, aphids caged on the scion leaves (*Mi-1*) started dying on day 2 and were all dead by day 7. In contrast, only one aphid died on the rootstock (*mi-1*) before day 7 (Fig. 5A). Similarly, on plants with the reciprocal chimeric grafts (*mi-1* scions grafted onto *Mi-1* rootstocks), over 85% of the potato aphids caged on the scion leaves (*mi-1*) were alive on day 7, when the experiment was terminated (Fig. 5B). In contrast, aphids caged on the rootstock leaves (*Mi-1*) started dying on day 1, and all aphids were dead by day 7 (Fig. 5B). On control plants, with *Mi-1/Mi-1* (scion/rootstock) grafts, potato aphids started dying on day 2 on both sides of the grafts, and all aphids were dead by day 7 (data not shown). As expected, on *mi-1/mi-1* (scion/rootstock) grafted plants, only small decreases in the number of viable aphids were seen on rootstock or scion leaves. Over 80% of the aphids were viable on the root stock leaves (*mi-1*) at day 6, when the experiment was terminated (data not shown).

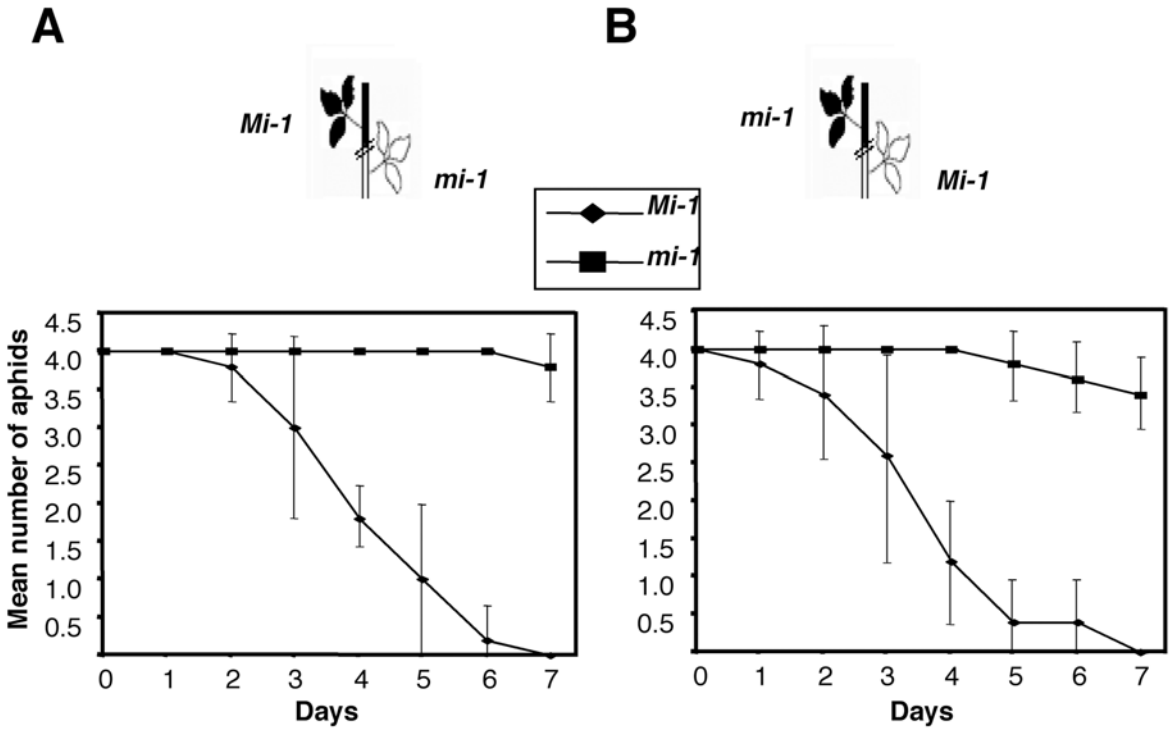

Fig. 5. *Mi-1*–mediated potato aphid resistance is not transmitted through a graft union. Near-isogenic tomato cvs. Sun6082 (*Mi-1*) and CastlerockII *(mi-1*) were used in reciprocal grafts. Top, schematic diagram of the reciprocal grafts. Bottom, survivorship of *Mi-1*–avirulent biotype of the potato aphid on chimeric plants. **A,** Chimeric plants when *Mi-1* tomato plants were used as scions on *mi-1* rootstocks. **B,** Chimeric plants when *mi-1* tomato plants were used as scions on *Mi-1* rootstocks. Day-old apterous aphids (4) were caged individually on either side of the graft unions, and aphid survivorship was recorded daily until all the aphids on the *Mi-1* portion of the graft union were dead. Four plants of each graft combination were used.

Maximum likelihood was used to estimate the probability of death (p) for each of the four resistant portions and each of the four susceptible portions of the grafted plants, as well as overall estimates for resistant and susceptible portions of the grafted plants. A test for the equality of the probability of death for aphids on the resistant portion of the grafted plants was not significant ($\chi^2 = 0.19$, $df = 3$, $p > 0.10$), indicating that there was no difference in the probability of death independent of whether the resistant portion of the plant was a scion or a rootstock. Similarly, a test for the equality of the probability of death for aphids on the susceptible portion of the plants was not significant ($\chi^2 = 1.00$, $df = 3$, $p > 0.10$), indicating that there was no difference in the probability of death independent of whether the susceptible portion of the plant was a scion or a rootstock. Collectively, these results indicated that the resistance mediated by *Mi-1*was not translocated across a graft union. Using pooled estimates of the probability of death on *Mi-1* ($p = 0.2374$) and *mi-1* ($p = 0.0236$) portions of the grafts indicated that there was a highly significant difference in the probability of death for resistant and susceptible portions of chimeric plants ($\chi^2 = 103.27$, $df = 1$, $p < 0.0001$).

DISCUSSION

Our results suggest that both JA and SA signal pathways were activated by aphid feeding. JA- and wound-regulated genes *Pin1* and *Pin2* were induced rapidly and transiently at 6 and 12 hai by aphid feeding. Earlier reports indicated that proteinase inhibitors were not induced in tomato by potato aphid feeding (Fidantsef et al. 1999; Stout et al. 1998). Possibly, the absence of proteinase inhibitor RNA detection in those studies was due to the time of tissue sampling (1 week postinfestation). Transcripts of the JA- and wound-inducible *LoxD* (Heitz et al. 1997) were detected in both incompatible and compatible interactions as well as in uninfested controls. These data were in agreement with an earlier report that *LOX* mRNAs were induced in susceptible tomato by potato aphid (Fidantsef et al. 1999). Similarly, induction of *LOX2* transcript levels by the green peach aphid has been observed in *Arabidopsis* (Moran and Thompson 2001). Although lipoxygenases have been shown to be induced in the race-specific resistance to *C. fulvum* mediated by the tomato *Cf-9* gene (Durrant et al. 2000), it is likely that the elevated levels of *LoxD* in controls and both incompatible and compatible interactions is caused by mechanical or aphid feeding injury, or both. Unlike chewing insects, most aphids penetrate plant cells delicately, moving their stylets between the cells, therefore minimizing injury. However, potato aphids feeding on tomato showed significant intracellular stylet penetration of epidermis and mesophyll cells (over 27% of the probes) and probed twice as frequently on resistant than on susceptible plants (Kaloshian et al. 2000). Nonetheless, it is possible that wound-response genes are also part of the *Mi-1*–mediated resistance, as considerable overlap in profiles of race-specific resistance and wound-response genes has been reported recently (Durrant et al. 2000).

Significant differences in *PR* gene expression were observed in the compatible and incompatible interactions of the potato aphid and tomato plants. For example, *PR-1* transcripts were observed earlier and accumulated to higher levels in the incompatible than in the compatible potato aphid interactions, indicating that *Mi-1*–mediated resistance to aphid may involve the SA signaling pathway. Faster accumulation of *PR* protein transcripts in incompatible interactions is well-documented in resistance gene-mediated defense pathways in tomato (van Kan et al. 1992; Jia and Martin 1999). However, not all *PR* proteins are induced earlier in incompatible interactions (van Kan et al. 1992). In the potato aphid-tomato studies presented here, *GluB* RNAs were detected at similar times and levels in both compatible and incompatible interactions. In contrast, Russian wheat aphid infestation caused both intracellular and intercellular β-1,3-glucanase activities (acidic and basic forms) to rise to higher levels at 48 h in resistant wheat plants than in susceptible plants (van der Westhuizen et al. 1998).

Both *PR-1* and *GluB* RNAs accumulated systemically in adjacent noninfested leaflets in Motelle (*Mi-1*) plants at 48 hai after potato aphid infestation (an incompatible interaction) and green peach aphid infestation (a compatible interaction). Neither aphid caused systemic expression of *PR-1* or *GluB* in Moneymaker plants (*mi-1*). Because the two tomato varieties are nearly isogenic, it is unlikely that the systemic induction of these genes is caused by the minor differences in their genetic background. Therefore, these data suggest that *Mi-1* influences the systemic responses to the two aphid species. The systemic induction of *PR-1* in Motelle (*Mi-1*) plants in both compatible (green peach aphid) and incompatible (potato aphid) interactions and more rapid accumulation of *PR-1* transcripts in the incompatible potato aphid interaction may indicate that *PR-1* is necessary but not sufficient for *Mi-1*–mediated resistance to potato aphid.

It is not clear whether the *Mi-1*–mediated resistance to root-knot nematodes involves the SA signal pathway. Although a number of tomato genes whose transcripts were upregulated by nematode infection have been identified, none of these genes are associated directly with the SA signaling pathway (Lambert et al. 1999). Interestingly, information exists about induced resistance to root-knot nematodes in tomato roots. Using a split root system, Ogallo and McClure (1996) showed that preinoculation of one half of the split root system of *Mi-1*–containing tomato with an avirulent population of *M. incognita* reduces the infection rate of the virulent nematode *M. hapla* in the other half (Ogallo and McClure 1996).

Our result supports earlier findings that aphid feeding-induced defense responses are activated by both SA and JA/ethylene pathways (Moran and Thompson 2001; Walling 2000). There are indications that early responses of *Mi-1*–mediated potato aphid resistance involve JA- and ethylene-induced pathways, and later responses involve SA- and ethylene-induced pathways. Cross talk between these pathways is complex and highly regulated with both antagonistic and synergistic effects (Bostock et al. 2001; Glazebrook 2001; Walling 2000). Recently, significant coordination of interactions between JA and SA pathways has been reported. Using microarray analysis, Schenk and associates (2000) showed that a large number of genes were coinduced or corepressed by these signaling molecules. It is possible that phloem-feeding insects elicit novel defense mechanisms and signal pathways in plants (Moran and Thompson 2001; van de Ven et al. 2000; Walling 2000). A more extensive and direct transcript expression display, reflecting the potato aphid *Mi-1*–mediated defense response, is required to identify the players in this response.

Transcripts of *PR-1*, *Pin1*, and *Pin2* were detected as early as 6 hai; however, H_2O_2 was not detected until 24 hai in tomato leaflets in both compatible and incompatible interactions. These results suggest that activation of oxidative burst does not precede accumulation of defense gene transcripts. The sensitivity of the DAB assay may be limited; therefore, we can not exclude the possibilities of the presence of micro oxidative bursts (Alvarez et al. 1998) or efficient scavenging of H_2O_2 by either plant catalases or peroxidases present in aphid saliva (Miles 1990).

Grafting experiments were performed to determine if *Mi-1.2*–mediated resistance to the potato aphid is transmitted systemically. These grafting experiments did not address the existence of systemic acquired resistance to aphids, since aphids

were caged on both sides of the graft union at the same time. The reciprocal grafting experiments demonstrated that the *Mi-1*–mediated resistance to aphids is not translocated through a graft union. Similar results have been reported in muskmelon (*Cucumis melo* L.) with resistance to the melon aphid *Aphis gossypii* Glover, when fewer aphids were observed on resistant than on susceptible plant portions, irrespective of the graft union (Kennedy and Kishaba 1977). Although no nematode or aphid avirulence factors have yet been identified, according to the gene-for-gene model, it is presumed that these factors interact directly or indirectly with *Mi-1* to trigger the defense response. Since the lines used are near isogenic and all the signal transduction components required for *Mi-1*–mediated resistance are present in susceptible tomato varieties (Milligan et al. 1998), the grafting experiments indicate that *Mi-1*–mediated resistance is cell autonomous and interaction between aphid elicitor and Mi-1 is required for resistance.

MATERIALS AND METHODS

Plant materials.

Five different tomato genotypes were used in this study, including two pairs of near-isogenic tomato. The first near-isogenic pair was Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*), and the second near-isogenic pair was Sun6082 (*Mi-1/Mi-1*) and CastlerockII (*mi-1/mi-1*). In addition, tomato cv. Rio Grande 76R (*Pto/Pto*) was used. Seeds were sown in seedling trays filled with organic planting mix and, three weeks after germination, were transplanted into plastic cups (10-cm diameter, 17 cm deep) filled with University of California mix II. Plants 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.). All plants were maintained in a greenhouse with temperatures ranging between 22 to 26°C.

Insect colonies.

A clonal colony of the parthenogenetic *Mi-1*–avirulent potato aphid (obtained from P. Piron, Plant Research International, Wageningen, The Netherlands) was maintained either on UC82 (*mi-1/mi-1*) tomato or potato plants in an insect cage in a greenhouse. Aphids maintained on potato plants were exposed to tomato seedlings for one week before use in experiments. A colony of the green peach aphid (obtained from B. Falk, University of California, Davis, U.S.A.) was grown on *Brassica* spp. plants in an insect cage.

Timecourse experiment for transcript analysis.

For each infestation experiment, 40 apterous mixed-stage aphids (adults and nymphs) were carefully removed from the infested plants, put into a clip cage, and secured onto the same leaflet on the fourth or fifth leaf of eight-week-old tomato plants. Clip cages were clipped onto leaflets so as to allow aphids to feed on the abaxial leaf surface. One cage was used per plant, two plants were infested for each timepoint, and tissue was pooled. With potato aphid infestation, infested leaflets were collected 6, 12, 24, and 48 h and 1 week after infestation. With green peach aphid infestations, infested leaflets were collected 6, 12, 24, and 48 hai. For analysis of systemic expression, the leaflet opposing the infested one was also collected at each timepoint. Control plants received empty clip cages, and leaflets were collected at each timepoint. Cages were removed and individual tomato leaflets were excised using a razor blade. Tissues were immediately frozen in liquid nitrogen and were stored at –80°C. Two independent experiments were performed.

RNA isolation and RNA blot analyses.

Total RNA was isolated as described by Martinez de Ilarduya and associates (2001). Total RNAs (15 μg per lane) were separated on a 1.2% agarose/formaldehyde gel and were transferred to a nylon membrane Nytran Super Charge (Schleicher & Schuell, Keene, NH, U.S.A.). All gels were stained with ethidium bromide to assure equal loading per lane. At least two different blots from each set of samples were prepared.

The cDNA clones *PR-1* (GenBank accession number M69247) and *GluB* (GenBank accession number M80608) were provided by P. de Wit (Wageningen University, The Netherlands), *Pin1* (GenBank accession number K03290) and *Pin2* (GenBank accession number K03291) were obtained from C. Ryan (Washington State University, Pullman, U.S.A.). The lipoxygenase (*LoxD)* (GenBank accession number U37840) was obtained by amplification of tomato genomic DNA with the primers LoxD-F (5′-CCATCTCTTATGGCCAGCAT-3′) and LoxD-R (5′-GTGACAACACGTTTGGATCG-3′). An 18S rRNA was used as a control of equal loading between lanes. DNA probes were labeled with ^{32}P-α-dCTP using the Rediprime 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, were hybridized for 16 h at 42°C, and were washed as described by Martinez de Ilarduya and associates (2001). To directly compare values from different blots, blots were hybridized in the same bottle, washed under the same conditions, and exposed to an Imaging Screen-K for the same length of time. Signals were quantified on a Molecular Imager FX System using the Quantity One analysis software (Bio-Rad, Hercules, CA, U.S.A.). Data were normalized using the signal obtained from the rRNA probe. For each gene, the highest normalized value was given a value of 1. RNA accumulation in the remaining timepoints was calculated relative to this value. Data represents two duplicated blots obtained from two independent experiments. The relative intensity of a probe at different timepoints was analyzed using one way ANOVA (SAS software release 6.12 1996).

After quantification, probes were stripped by pouring boiling 0.5% sodium dodecyl sulfate on the membrane and allowing it to cool to room temperature. Blots were checked for complete removal of the probe and were reused. Typically, up to six probes were used on the same blot. The rRNA probe was always used last.

Histological analyses.

Tomato leaflets were infested with aphids similar to the RNA blot experiments, except 10 to 15 potato aphids (mixed stages) were used per cage. Control leaflets were clipped with empty cages. Samples were collected at 3, 6, 12, and 24 hai. As a positive H_2O_2 control, tomato leaflets were mechanically injured with a hemostat and were immediately used in the H_2O_2 detection assay. H_2O_2 was detected using 3,3'-diamaminobenzidine (DAB) (Sigma, St. Louis) as substrate (Thordal-Christensen et al. 1997). Control tomato leaf disks (2-cm diameter) and potato aphid-infested leaf disks were vacuum-infiltrated with 1 mg ml^{-1} solution of DAB, pH 3.8, for 20 min. Leaf disks were incubated in DAB solution for an additional 6 h at room temperature. Experiments were terminated by immersing the leaf disks in boiling 95% ethanol to clear the tissue, and the disks were stored in 70% ethanol at room temperature. H_2O_2 was visualized as a reddish brown coloration. This experiment was repeated twice.

Trypan blue staining for cell viability and cell death detection was performed according to Rate and associates (1999). Tomato leaflets were infested as described for the H_2O_2 detection. Samples were collected at 12, 24, and 48 hai. Tomato leaf

disks (2-cm diameter) were boiled in a solution of equal parts of phenol, lactic acid, glycerol, and water and 0.05% (wt/vol) trypan blue for 2 min. Leaf disks were destained by boiling in lactophenol glycerol solution without trypan blue for 5 min. Disks were rinsed and kept in 70% ethanol at room temperature. The experiment was repeated twice.

Acid fuchsin was used to stain for aphid salivary sheath (Backus et al. 1988). Tomato leaflets were infested and collected as for trypan blue staining. Tomato leaf disks (2-cm diameter) were stained overnight in a solution of 0.1% acid fuchsin in equal parts of 95% ethanol and glacial acetic acid. Tissue was destained in a solution of equal parts of lactic acid, glycerol, and water by autoclaving for 10 min. This experiment was repeated twice. Both trypan blue- and acid fuchsin-stained material were visualized using differential interference contrast optics. Images were captured using a SpotRT digital camera (Diagnostics Instruments, Sterling Heights, MI, U.S.A.)

Grafting and aphid infestation.

Near-isogenic tomato plants resistant (Sun6082) and susceptible (CastlerockII) to the potato aphid were used for grafting. Four-week-old plants were used as scions and stock. To prepare for scions, plants were cut above the second fully expanded leaf, and most of the leaflets were removed to minimize transpiration. The stem was cut in a V-shape, using a sharp blade. The plant used as a stock was decapitated at the second fully expanded leaf, and a slit was formed in its stem. The scion was inserted in the slit of the stock, and the graft junction was held together by wrapping parafilm tightly around the union. Plants were kept in a room with low lights and were sprayed with water twice a day for four to five days. Plants were moved to a greenhouse for four weeks.

Age-synchronized aphids were reared as described previously (Kaloshian et al. 1997). One- to two-day-old apterous adult aphids were used for this study. Single aphids were individually caged on the abaxial leaf surface of four leaflets of the scion and four leaflets of the stock of individual grafted plants. Four plants of each of the scion/root stock graft combinations were infested and were maintained in a greenhouse. Aphids were monitored every 24 h, until all aphids on the resistant genotype plants died.

Statistical analysis.

The data generated from the grafting experiment was analyzed as a series of dependent binomial experiments with a constant probability of death on any given day equal to p. The number of aphids on test on the scion portion of the plant is n, and the number of aphids on test on the rootstock portion of a plant is also n. Each scion and rootstock is treated independently of the others. For a given scion or rootstock, the number of aphids who die on day 1 is denoted by x_1. On day 2, the number of aphids on test is $n - x_1$, and the number of aphids that die is x_2. Similarly, the number of aphids on test on day i is $n - x_1 - \ldots - x_{i-1}$, and the number of aphids that die is x_i, such that $i = 1, \ldots, 7$. Maximum likelihood was used to estimate the probability of death, p, for each of the four resistant portions and four susceptible portions of the grafted plants, as well as overall estimates for resistant and susceptible portions of the plants.

ACKNOWLEDGMENTS

We thank R. Beaver for statistical analysis, S. Edwards for help with figures, and L. Walling, M. Orozco-Cardenas, and J. Smith-Becker for critically reading the manuscript. This research was supported by a grant to I. Kaloshian from the U. S. Department of Agriculture National Research Initiative Competitive Grants Program (No 2000-02876).

LITERATURE CITED

Alvarez, M. E., Pennell, R. I., Meijer, P.-J., Ishikawa, A., Dixon, R. A., and Lamb, C. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92:773-784.

Backus, E. A., Hunter, W. B., and Arne, C. N. 1988. Technique for staining leafhopper (Homoptera: Cicadellidae) salivary sheath and eggs within unsectioned plant tissue. J. Econ. Entomol. 81:1819-1823.

Bostock, R., Karban, R., Thaler, J., Weyman, P., and Gilchrist, D. 2001. Signal interactions in induced resistance to pathogens and insect herbivores. Eur. J. Plant Pathol. 107:103-111.

Chao, W., Gu, Y., Pautot, V., Bray, E., and Walling, L. 1999. Leucine aminopeptidase RNAs, proteins, and activities increase in response to water deficit, salinity, and the wound signals systemin, methyl jasmonate, and abscisic acid. Plant Physiol. 120:979-992.

Durrant, W., Rowland, O., Piedras, P., Hammond-Kosack, K., and Jones, J. 2000. cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. Plant Cell 12:963-977.

Ellis, C., Karafyllidis, I., and Turner, J. G. 2002. Constitutive activation of jasmonate signaling in an *Arabidopsis* mutant correlates with enhanced resistance to *Erysiphae cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. Mol. Plant-Microbe Interact. 15:1025-1030.

Fidantsef, A. L., Stout, M. J., Thaler, J. S., Duffey, S. S., and Bostock, R. M. 1999. Signal interactions in pathogen and insect attack: Expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. Physiol. Mol. Plant Pathol. 54:97-114.

Forslund, K., Pettersson, J., Bryngelsson, T., and Jonsson, L. 2000. Aphid infestation induces PR-proteins differently in barley susceptible or resistant to the birdcherry-oat aphid (*Rhopalosiphum padi*). Physiol. Plant 110:496-502.

Glazebrook, J. 2001. Genes controlling expression of defense responses in *Arabidopsis*—2001 status. Curr. Opin. Plant Biol. 4:301-308.

Goggin, F. L., Williamson, V. M., and Ullman, D. E. 2001. Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene *Mi*. Environ. Entomol. 30:101-106.

Graham, J. S., Pearce, G., Merryweather, J., Titani, K., Ericsson, L. H., and Ryan, C. A. 1985a. Wound-induced proteinase inhibitors from tomato leaves. I. The cDNA-deduced primary structure of pre-inhibitor I and its post-translational processing. J. Biol. Chem. 260:6555-6560.

Graham, J. S., Pearce, G., Merryweather, J., Titani, K., Ericsson, L. H., and Ryan, C. A. 1985b. Wound-induced proteinase inhibitors from tomato leaves. II. The cDNA-deduced primary structure of pre-inhibitor II. J. Biol. Chem. 260:6561-6564.

Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W., and Dangl, J. L. 1995. Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. Science 269:843-846.

Heitz, T., Bergey, D. R., and Ryan, C. A. 1997. A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate. Plant Physiol. 114:1085-1093.

Jia, Y., and Martin, G. 1999. Rapid transcript accumulation of pathogenesis-related genes during an incompatible interaction in bacterial speck disease-resistant tomato plants. Plant Mol. Biol. 40:455-465.

Kaloshian, I., Lange, W. H., and Williamson, V. M. 1995. An aphid resistance locus is tightly linked to the nematode resistance gene, *Mi,* in tomato. Proc. Natl. Acad. Sci. U.S.A. 92:622-625.

Kaloshian, I., Williamson, V. M., Miyao, G., Lawn, D. A., and Westerdahl, B. B. 1996. Identification of "resistance breaking" field populations of root-knot nematodes on tomato in California. Cal. Agri. 50:18-20.

Kaloshian, I., Kinsey, M. G., Ullman, D. E., and Williamson, V. M. 1997. The impact of *Meu1*-mediated resistance in tomato on longevity, fecundity and behavior of the potato aphid, *Macrosiphum euphorbiae*. Entomol. Exp. Appl. 83:181-187.

Kaloshian, I., Kinsey, M. G., Williamson, V. M., and Ullman, D. E. 2000. *Mi*-mediated resistance against the potato aphid, *Macrosiphum euphorbiae* (Hemiptera: Aphididae) limits sieve element ingestion. Environ. Entomol. 29:690-695.

Kennedy, G. G., and Kishaba, A. N. 1977. Response of alate melon aphids to resistant and susceptible muskmelon lines. J. Econ. Entomol. 70:407-410.

Kim, Y. J., Lin, N.-C., and Martin, G. B. 2002. Two distinct *Pseudomonas* effector proteins interact with Pto kinase and activate plant immunity. Cell 109:589-598.

Krishnaveni, S., Muthukrishnan, S., Liang, G., Wilde, G., and Manickam, A. 1999. Induction of chitinases and beta-1,3-glucanases in resistant and susceptible cultivars of sorghum in response to insect attack, fun-

gal infection and wounding. Plant Science 144:9-16.
Lamb, C., and Dixon, R. A. 1997. The oxidative burst in plant disease resistance. Annu. Rev. Physiol. Plant Mol. Biol. 48:251-275.
Lambert, K., Ferrie, B., Nombela, G., Brenner, E., and Williamson, V. 1999. Identification of genes whose transcripts accumulate rapidly in tomato after root-knot nematode infection. Physiol. Mol. Plant Pathol. 55:341-348.
Martinez de Ilarduya, O., and Kaloshian, I. 2001. *Mi-1.2* transcripts accumulate ubiquitously in root-knot nematode resistant *Lycopersicon esculentum*. J. Nematol. 33:116-120.
Martinez de Ilarduya, O., Moore, A. E., and Kaloshian, I. 2001. The tomato *Rme1* locus is required for *Mi-1*-mediated resistance to root-knot nematodes and the potato aphid. Plant J. 27:417-425.
Miles, P. W. 1999. Aphid saliva. Biol. Rev. 74:41-85.
Milligan, S. B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P., and Williamson, V. M. 1998. The root-knot nematode gene *Mi* from tomato is a member of the leucine zipper-nucleotide binding leucine-rich repeat family of plant genes. Plant Cell 10:1307-1320.
Moran, P. J., and Thompson, G. A. 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. Plant Physiol. 125:1074-1085.
Niderman, T., Genetet, I., Bruyere, T., Gees, R., Stintzi, A., Legrand, M., Fritig, B., and Mosinger, E. 1995. Pathogenesis-related PR-1 proteins are antifungal—Isolation and characterization of three 14-kilodalton proteins of tomato and of basic PR-1 of tobacco with inhibitory activity against *Phythophthora infestans*. Plant Physiol. 108:17-27.
Ogallo, J. L., and McClure, M. A. 1996. Systemic acquired resistance and susceptibility to root-knot nematodes in tomato. Phytopathology 86:498-501.
Orozco-Cardenas, M., and Ryan, C. A. 1999. Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proc. Natl. Acad. Sci. U.S.A. 96:6553-6557.
Penninckx, I., Thomma, B., Buchala, A., Metraux, J.-P., and Broekaert, W. 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10:2103-2113.
Rate, D. N., Cuenca, J. V., Bowman, G. R., Guttman, D. S., and Greenburg, J. T. 1999. The gain-of-function *Arabidopsis acd6* mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. Plant Cell 11:1695-1708.
Rossi, M., Goggin, F. L., Milligan, S. B., Kaloshian, I., Ullman, D. E., and Williamson, V. M. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc. Natl. Acad. Sci. U.S.A. 95:9750-9754.
Schenk, P., Kazan, K., Wilson, I., Anderson, J., Richmond, T., Somerville, S., and Manners, J. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc. Natl. Acad. Sci. U.S.A. 97:11655-11660.
Smith, P. G. 1944. Embryo culture of a tomato species hybrid. Proc. Am. Soc. Hortic. Sci. 44:413-416.
Stout, M., Workman, K., Bostock, R., and Duffey, S. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. Oecologia 113:74-81.
Thomma, B., Eggermont, K., Broekaert, W., and Cammue, B. 2000. Disease development of several fungi on *Arabidposis* can be reduced by treatment with methyl jasmonate. Plant Physiol. Biochem. 38:421-427.
Thomma, B., Penninckx, I., Broekaert, W., and Cammue, B. 2001. The complexity of disease signaling in *Arabidopsis*. Curr. Opin. Immunol. 13:63-68.
Thordal-Christensen, H., Zhang, Z., Wei, Y., and Collinge, D. B. 1997. Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. Plant J. 11:1187-1194.
van de Ven, W., LeVesque, C., Perring, T., and Walling, L. 2000. Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. Plant Cell 12:1409-1423.
van der Westhuizen, A., Qian, X., and Botha, A. 1998. β-1,3-glucanases in wheat and resistance to the Russian wheat aphid. Physiol. Plant. 103:125-131.
van Kan, J., Cozijnsen, T., Danhash, N., and de Wit, P. 1995. Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. Plant Mol. Biol. 27:1205-1213.
van Kan, J., Joosten, M., Wagemakers, C., van den Berg-Velthuis, G., and de Wit, P. 1992. Differential accumulation of messenger RNAs encoding extracellular and intracellular proteins in tomato induced by virulent and avirulent races of *Cladopsorium fulvum*. Plant Mol. Biol. 20:513-527.
Walling, L. 2000. The myriad plant responses to herbivores. J. Plant Growth Regul. 19:195-216.