Oxylipins, which encompass a large family of oxidized fatty acids, play a pivotal role as signaling molecules and protective compounds in plant response to biotic stress (Blee, 2002; Prost et al., 2005). Oxylipins are also implicated in cross-kingdom communication between plants and pathogenic fungi (Christensen and Kolomiets, 2011). The first step in the synthesis of oxylipins involves the formation of fatty acid hydroperoxides either by autoxidation or by the action of enzymes, such as lipoxygenases (LOXs) and α-dioxygenases (Feussner and Wasternack, 2002; Mosblech et al., 2009). Spontaneous or enzymatic modification of fatty acid hydroperoxides yields an additional array of oxylipins (Feussner and Wasternack, 2002; Mosblech et al., 2009).

The Arabidopsis thaliana genome contains six LOX genes, which encode proteins that are classified as 9- and 13-LOXs based on their ability to incorporate oxygen either at the C-9 or C-13 position of the fatty acid, yielding the 9- or 13-fatty acid hydroperoxides, respectively (Livanonchanka and Feussner, 2006). Arabidopsis LOX1 and LOX5 encode 9-LOXs, whereas LOX2, LOX3, LOX4, and LOX6 encode 13-LOXs (Bannenberg et al., 2009). Jasmonic acid (JA), which is derived from the 13-LOX pathway, is one of the best-studied oxylipins that has a signaling function in plant growth and development and in stress response (Hove and Jander, 2008; Browse, 2009; Wu and Baldwin, 2010). 9-LOX–derived oxylipins are also involved in plant growth and development and in stress response. For example, 9-LOX–derived oxylipins are involved in lateral root development in Arabidopsis and maize (Zea mays) (Vellosillo et al., 2007; Gao et al., 2008) and in defense against bacterial pathogens in pepper (Capsicum annuum) and Arabidopsis (Vellosillo et al., 2007; Hwang and Hwang, 2010; López et al., 2011) and against fungal pathogens and nematodes in maize (Gao et al., 2007; Gao et al., 2008; Gao et al., 2009).

Insect infestation is a major factor that limits plant production (Ferry et al., 2006). Insect pests of plants can be classified into two broad groups, the chewing insects and the piercing/sucking insects. Chewing insects use their strong mandibles to chew on plant tissue, thus resulting in extensive physical damage and tissue loss (Karban and Baldwin, 1997; Kandoth et al., 2007). Among the piercing/sucking insects, thrips and spider mites are cell-content feeders that use their mouthparts, which are modified into stylets, to consume the content of mesophyll and/or epidermal cells (Wallig, 2000). By contrast, aphids, whiteflies, and leafhoppers use their stylets to consume fluids from the vasculature. This feeding behavior of aphids and whiteflies results in minimal wounding to the plant (Wallig, 2000).

More than 250 species of aphids are pests of plants (Dixon, 1998; Blackman and Eastop, 2000). Aphids affect plant productivity by removal of photoassimilates and by altering source-sink patterns (Dixon, 1998; Blackman and Eastop, 2000; Goggin, 2007). In addition, some aphids are also vectors for plant viruses (Kennedy et al., 1962; Matthews, 1991; Guerrieri and Digilio, 2008). Although the aphid stylet penetrates the host tissue primarily intercellularly, occasionally it punctures cells, allowing the insect to sample cell contents (Pollard, 1973). Two types of saliva are released by aphids into the plant. The gelling saliva, which is secreted when the stylet is penetrating host tissue, insulates the stylet from plant tissue and forms a tight seal around the penetrated site. By contrast, the watery saliva, which contains several hydrolytic enzymes and is intermittently released into the sieve elements during insect feeding from the phloem (Miles, 1999; Tjallingii, 2006), likely aids in reversal of phloem

**INTRODUCTION**

Oxylipins function as signaling molecules in plant growth and development and contribute to defense against stress. Here, we show that oxylipins also facilitate infestation of Arabidopsis thaliana shoots by the phloem sap–consuming green peach aphid (GPA; Myzus persicae), an agronomically important insect pest. GPAs had difficulty feeding from sieve elements and tapping into the xylem of lipoxygenase5 (lox5) mutant plants defective in LOX activity. These defects in GPA performance in the lox5 mutant were accompanied by reduced water content of GPAs and a smaller population size of GPAs in the mutant compared with the wild-type plant. LOX5 expression was rapidly induced in roots in response to infestation of shoots by GPAs. In parallel, levels of LOX5-derived oxylipins increased in roots and in petiole exudates of GPA-colonized plants. Application of 9-hydroxyoctadecadienoic acid (an oxylipin produced by the LOX5 enzyme) to roots restored water content and GPA population size in lox5 plants, thus confirming that a LOX5-derived oxylipin promotes infestation of the foliage by GPAs. Micrografting experiments demonstrated that GPA performance on foliage is influenced by the LOX5 genotype in roots, thus demonstrating the importance of root-derived oxylipins in colonization of aboveground organs by an insect.

1 Address correspondence to Shah@unt.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Jyoti Shah (Shah@unt.edu).

© 2012 American Society of Plant Biologists. All rights reserved.

The Plant Cell Preview, www.aspb.org 2012 American Society of Plant Biologists. All rights reserved.

1 of 11
occlusion (Will et al., 2007), thus facilitating continued feeding from the sieve elements. The sugar-rich phloem sap that is consumed by aphids has an osmotic potential that is considerably more negative than the aphid hemolymph, and the resulting gradient could result in the dehydration of the aphid (Douglas, 2006). Occasionally, aphids also ingest xylem sap (Spiller et al., 1980; Tjallingii and Hogen Esch, 1993; Powell and Hardie, 2002; Douglas, 2006). The lower solute concentration in xylem sap and hence a higher osmotic potential as compared with phloem sap (Mattson, 1980; Taiz and Zeiger, 2002) is suggested to enable aphids to osmoregulate their hemolymph and prevent dehydration (Pompon et al., 2010, 2011).

Some aphids have a restricted host range and are considered as specialists. For example, the host range of cabbage aphid (Brevicoryne brassicae) and mustard aphid (Lipaphis erysimi) is limited to related cruciferous species (Blackman and Eastop, 2000). By contrast, a generalist aphid like Myzus persicae Sulzer, more commonly known as the green peach aphid (GPA), is polyphagous and has a wide host range that includes more than 50 plant families (Blackman and Eastop, 2000). It has been suggested that generalist aphids use nutritional cues to make their host selections (Powell et al., 2006), whereas specialist aphids utilize plant secondary metabolites as cues for host recognition, feeding, and oviposition (Raybould and Moyes, 2001; Macel and Vrieling, 2003).

The coevolution of plants and their insect pests has resulted in the development of complex interactions between them. Whereas plants have evolved a variety of defense and tolerance mechanisms against insect herbivory (Howe and Jander, 2008), insects have evolved counterstrategies to bypass and/or overcome the barriers used by plants (Hogenhout and Bos, 2011). Much of our current understanding of molecular mechanisms that influence plant interaction with insects arises from studies dealing with response of the aboveground shoot foliage to insect attack (Howe and Jander, 2008). However, although less well studied, physiological changes in the roots also affect the severity of infestation in the shoots (Erb et al., 2009). For example, a variety of secondary metabolites (e.g., nicotine) that are involved in protecting aboveground tissues are synthesized in roots, from which they are transported to shoots in response to insect infestation (Baldwin et al., 1994; Erb et al., 2009; Morita et al., 2009). Here we show that in Arabidopsis, the LOX5-encoded 9-LOX–derived oxylipin(s) functions as a susceptibility factor in plant interaction with GPA. LOX5 facilitates insect feeding from sieve elements and water consumption from xylem. We provide genetic and biochemical evidence that roots are the source of the LOX5-derived oxylipin(s) that facilitates colonization of Arabidopsis shoots by GPA, thus highlighting the dependence of GPA on root-derived oxylipins for colonizing Arabidopsis.

RESULTS

The GPA Population Size Is Smaller in lox5 Mutant Plants Than in Wild-Type Arabidopsis Plants

Accumulation of 9-LOX–synthesized oxylipins and volatile compounds derived from these oxylipins was previously shown to be stimulated in leaves of plants infested with aphids (Gosset et al., 2009). Some 9-LOX–derived volatiles induce oviposition in aphid predators (Verheggen et al., 2008), thus implicating a potential role for 9-LOXs in indirect defense against aphids. 9-LOX-synthesized oxylipins also accumulate both in phloem sap and in aphids feeding on plants (Harmel et al., 2007), suggesting that these oxylipins may also have a more direct role in plant–aphid interaction. Arabidopsis contains two 9-LOX–encoding genes, LOX1 (At1g55020) and LOX5 (At3g22400) (Bannenberg et al., 2009). Whereas LOX1 is localized to the plastids (see Supplemental Figures 1C, 1G, and 1K online), LOX5 exhibits extraplastidic localization (see Supplemental Figures 1D, 1H, and 1L online).

To determine whether either LOX1 or LOX5 has a role in plant–aphid interaction, a no-choice bioassay was conducted in which 20 adult apterous (wingless) GPAs were released both on wild-type Arabidopsis plants (accession Columbia-0 [Col-0]) and on lox1 or lox5 mutant plants that lack LOX1 or LOX5 function, respectively. Two days after inoculation, the size of the insect population on each plant was determined. As shown in Figure 1A, GPA population size on the Arabidopsis lox5-1 and lox5-3 mutants, which contain T-DNA insertions within the LOX5 coding sequence (see Supplemental Figures 2A to 2C online), was significantly smaller than in wild-type plants. Ectopic expression of LOX5 or a LOX5-green fluorescent protein (GFP) protein fusion from the Cauliflower mosaic virus 35S promoter attenuated the lox5-1– and lox5-3–conferred enhanced resistance to GPA (Figure 1B), thus confirming that some LOX5-dependent activity is a susceptibility factor that predisposes Arabidopsis to infestation by GPA. In comparison with the lox5 mutants, insect numbers in the lox1-1 and lox1-3 mutants were greater and comparable with those in the wild type (Figure 1A). Furthermore, in comparison with the wild type and lox1-1 single mutant, insect numbers were comparably low on the lox1-1 lox5-1 double mutant and the lox5-1 single mutant plant (Figure 1C), thus indicating that unlike LOX5, LOX1 does not have a discernible role in the Arabidopsis–GPA interaction.

Vascular Sap from GPA-Infested lox5 Mutant Plants Lacks an Activity That Facilitates GPA Infestation

Vascular sap–enriched petiole exudates of uninfested Arabidopsis contain an inhibitory activity that limits the size of the GPA population on an artificial diet (Figure 2) (Pegadaraju et al., 2007; Louis et al., 2010a; Louis et al., 2010b). However, by contrast with petiole exudates from leaves of uninfested plants, petiole exudates from leaves of GPA-infested wild-type plants increased the insect population size on an artificial diet (Figure 2), thus suggesting that GPA infestation manipulates the host to make vascular sap more suitable for the insect. Although LOX5 did not affect the accumulation of this infestation-promoting activity in petiole exudates from uninfested leaves (Figure 2), petiole exudates collected from GPA-infested leaves of the lox5-1 and lox5-3 mutants were less effective in promoting GPA population than comparable petiole exudates from wild-type leaves (Figure 2). We thus conclude that LOX5 is required for the GPA-infestation–associated alterations of vascular sap that make the sap more palatable to the insect.
Electrical penetration graph (EPG) analysis provides a sensitive tool to study insect feeding behavior in plants (Walker, 2000). Previously, EPG has been used to compare the effect of wild-type and mutant Arabidopsis genotypes on GPA feeding behavior (Pegadaraju et al., 2007; Louis et al., 2010a; Louis et al., 2010b; Singh et al., 2011). In EPG, a wired insect that is part of a low-voltage circuit is released on a plant, and its activity is electrically monitored. The pattern of different waveforms generated provides insights into the time spent by the insect in different activities, including the nonprobing phase (NP), when the insect stylet is not inserted into the plant, the pathway phase, when the insect stylet, although inserted in the plant tissue, is outside the phloem and xylem, the xylem-phase (XP), when the insect is drinking from the xylem, and the sieve-element phase (SEP), when the stylet is in the sieve element.

Comparisons of GPA feeding behavior in the wild-type and lox5-1 mutant plants revealed differences in the time spent by GPAs in SEP. As shown in Table 1, GPAs spent less time in SEP in lox5-1 mutant plants than in wild-type plants. GPAs also had difficulty tapping into the xylem of the lox5-1 mutant plant compared with the wild-type plant. Although 50% of the insects feeding on wild-type Arabidopsis exhibited one or more XP, no XP was observed in more than 100 h of EPG recording involving 16 insects on the lox5-1 mutant plant (Table 1). The reduced time spent by GPAs in SEP and XP on the lox5-1 mutant was paralleled by an increase in NP on the lox5-1 mutant compared with the wild-type plant. These results suggest that a product of LOX5 activity facilitates GPA feeding from sieve elements and water consumption from xylem.

To determine whether the absence of XP in GPAs reared on the lox5 mutant affects the hydration status of insects, water content was evaluated in aphids collected from the wild-type and lox5-1 and lox5-3 mutant plants. As shown in Figure 3, the percentage water content of GPAs reared on wild-type plants was significantly higher than in GPAs collected from lox5-1 and lox5-3 mutant plants. By contrast with insects reared on the lox5-1 and lox5-3 mutant, water content in GPAs reared on the lox5-1 and lox5-3 plants expressing either the 35S:LOX5 or 35S:LOX5-GFP transgenes was comparable with that in insects reared on the wild-type plant (Figure 3). These results indicate that the inability of GPA to tap into the xylem of the lox5 mutants is associated with lower water content in the insect. We therefore conclude that a LOX5-dependent factor(s) facilitates the insect’s ability to tap into xylem and phloem.

**LOX5 Function in Arabidopsis Roots Promotes Insect Performance in Leaves**

A survey of publicly available microarray data sets (http://www.genevestigator.com/) and quantitative RT-PCR experiments (see Supplemental Figure 3 online) revealed that LOX5 expression in Arabidopsis leaves was very poor. Furthermore, LOX5 expression was not significantly altered in GPA-infested leaves (see Supplemental Figure 3 online). Considering that GPA is unable to tap into xylem of the lox5 mutant and that the general flow of xylem contents is from roots to shoots, we hypothesized

![Figure 1](image1.png)

**GPA Population Size Is Smaller on the lox5 Mutants Than the Wild-Type Plant**

(A) No-choice test. Comparison of total numbers of GPAs (adults + nymphs) on the wild type (WT) and 9-LOX mutants, lox1-1, lox1-3, lox5-1, and lox5-3.

(B) No-choice test. Comparison of total number of GPAs on the wild type, lox5-1, lox5-3, and two independent 35S:LOX5 and 35S:LOX5-GFP transgenic lines each in the lox5-1 and lox5-3 backgrounds.

(C) No-choice test. Comparison of GPA numbers on the lox1-1 and lox1-1 single mutants and the lox1-1lox5-1 double mutant.

In (A) to (C), all values are means from 12 plants ± SE. Different letters above the bars indicate values that are significantly different (P < 0.05) from each other by GLM/ANOVA.

**Table 1. EPG: Time Spent by GPAs for Various Activities on Arabidopsis Wild-Type and lox5-1 Plants**

<table>
<thead>
<tr>
<th>Insect Activity</th>
<th>Wild Type</th>
<th>lox5-1</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time spent in NPa</td>
<td>1.89 ± 0.39</td>
<td>3.08 ± 0.44</td>
<td>0.055a</td>
</tr>
<tr>
<td>Time spent in pathway phaseb</td>
<td>4.63 ± 0.44</td>
<td>4.26 ± 0.48</td>
<td>0.678</td>
</tr>
<tr>
<td>Time spent in SEP</td>
<td>1.29 ± 0.29</td>
<td>0.66 ± 0.12</td>
<td>0.056b</td>
</tr>
<tr>
<td>Time spent in XPa</td>
<td>0.19 ± 0.06</td>
<td>n.d.</td>
<td>0.910b</td>
</tr>
<tr>
<td>Time to first probea</td>
<td>0.19 ± 0.05</td>
<td>0.16 ± 0.04</td>
<td>0.122</td>
</tr>
<tr>
<td>Time to first SEP</td>
<td>2.78 ± 0.52</td>
<td>1.93 ± 0.38</td>
<td>0.056b</td>
</tr>
<tr>
<td>Time to first XPa</td>
<td>4.58 ± 0.84</td>
<td>n.d.</td>
<td>0.910b</td>
</tr>
</tbody>
</table>

EPG recording was conducted on 16 aphids on plants of each genotype. Recording with each insect was conducted for 8 h. n.d., none detected.

aEach value in hours is the average ± SE of 16 replications.

bParameters that are significantly different (Kruskal-Wallis test) between GPAs reared on the wild type versus lox5-1 mutant.
Because the lox5 allele affected the amount of time spent in SEP and XP by GPAs and also the accumulation of an activity in petiole exudates that is associated with an increase in GPA population size (Table 1, Figure 2), 9-HP levels were also determined in petiole exudates collected from leaves of GPA-infested plants, and as controls, uninfested wild-type and lox5-1 mutant plants. As shown in Figure 5A, levels of these 9-HPs were elevated in petiole exudates collected from GPA-infested leaves of wild-type plants. However, the accumulation of these 9-HPs was attenuated in petiole exudates collected from GPA-infested leaves of the lox5-1 mutant. To determine whether one or more of these 9-HPs were capable of promoting GPA fecundity, GPA was cultivated on an artificial diet containing either 9-HOD, 9-HHOD, 9-HOT, or 9-HPOT. As shown in Figure 5B, in comparison with control diets, a diet containing ethanol (0.1%) that was used to dissolve the 9-HPs, insect numbers were higher on 9-HOD- and 9-HHOD-supplemented diets, confirming that some 9-LOX products enhance GPA population size. 9-HOT and 9-HPOT did not enhance insect numbers at the concentrations used. Quite the contrary, 9-HOT adversely affected insect numbers in this assay. Irrigation of the lox5-1 plants with 9-HOD

9-LOX Products Accumulate at Elevated Levels in Roots and Petiole Exudates of GPA-infested Plants

Because LOX5 encodes a 9-LOX (Bannenberg et al., 2009), we monitored levels of the following 9-LOX synthesized 9-hydroperoxy- and 9-hydroxy-fatty acids (9-HPs) in roots of wild-type and lox5-1 mutant plants in which shoots were colonized by GPA: 9-hydroxyoctadecadienoic acid (9-HOD), 9-hydroperoxyoctadecadienoic acid (9-HHOD), 9-hydroxyoctadecatrienoic acid (9-HOT), and 9-hydroperoxyoctadecatrienoic acid (9-HPOT). As shown in Figure 4C, levels of all four 9-HPs were higher in roots of GPA-infested wild-type plants compared with roots of uninfested wild-type plants. By contrast, in roots of GPA-infested lox5-1 mutant, this increase in 9-HPs was significantly lower, confirming that LOX5 contributes to the increase in 9-HPs in roots of GPA-infested plants.

Because the lox5-1 allele affected the amount of time spent in SEP and XP by GPAs and also the accumulation of an activity in petiole exudates that is associated with an increase in GPA population size (Table 1, Figure 2), 9-HP levels were also determined in petiole exudates collected from leaves of GPA-infested plants, and as controls, uninfested wild-type and lox5-1 mutant plants. As shown in Figure 5A, levels of these 9-HPs were elevated in petiole exudates collected from GPA-infested leaves of wild-type plants. However, the accumulation of these 9-HPs was attenuated in petiole exudates collected from GPA-infested leaves of the lox5-1 mutant. To determine whether one or more of these 9-HPs were capable of promoting GPA fecundity, GPA was cultivated on an artificial diet containing either 9-HOD, 9-HHOD, 9-HOT, or 9-HPOT. As shown in Figure 5B, in comparison with control diets, a diet containing ethanol (0.1%) that was used to dissolve the 9-HPs, insect numbers were higher on 9-HOD- and 9-HHOD-supplemented diets, confirming that some 9-LOX products enhance GPA population size. 9-HOT and 9-HPOT did not enhance insect numbers at the concentrations used. Quite the contrary, 9-HOT adversely affected insect numbers in this assay. Irrigation of the lox5-1 plants with 9-HOD

9-LOX Products Accumulate at Elevated Levels in Roots and Petiole Exudates of GPA-infested Plants

Because LOX5 encodes a 9-LOX (Bannenberg et al., 2009), we monitored levels of the following 9-LOX synthesized 9-hydroperoxy-
facilitated GPA infestation of the shoot (Figure 6A) and restored water content in insects collected from these plants (Figure 6B), thus confirming that a 9-LOX–derived oxylipin synthesized in roots facilitates infestation of the shoots by GPA.

**DISCUSSION**

Oxylipins are known for their participation in plant defense against biotic stress as antibiotic factors and as defense-stimulating signal molecules (Bléé, 2002; Prost et al., 2005). Our results indicate that a 9-LOX–derived oxylipin, or a product thereof, is a susceptibility factor that facilitates the colonization of *Arabidopsis* by GPAs. We have shown that GPA infestation of the foliage results in the upregulation of *LOX5* expression in roots (Figure 4A). This was paralleled by an increase in levels of 9-HPs in roots and petiole exudates (Figures 4C and 5A). *LOX5* was required for this increase in content of 9-HPs in GPA-infested plants (Figures 4C and 5A). In agreement with a role of *LOX5* in plant susceptibility to GPA, in a no-choice assay, GPA population size was significantly smaller on the *lox5* mutants than on the wild-type plant (Figures 1A to 1C). Expression of *LOX5* or a *LOX5*-GFP fusion from the 35S promoter and exogenous application of the *LOX5*-derived oxylipin, 9-HOD, complemented the GPA infestation by GPAs.
Arabidopsis GPA infestation in oxylipin(s), or product thereof, has an important role in facilitating (Figures 1B and 6A), thus confirming that a LOX5-synthesized lox5 deficiency, resulting in the promotion of insect infestation (Figures 1B and 6A), thus confirming that a LOX5-synthesized oxylipin(s), or product thereof, has an important role in facilitating GPA infestation in Arabidopsis.

Aphid infestation is known to manipulate host physiology, leading to alterations in the composition of phloem sap (Sandström et al., 2000; Dinant et al., 2010; Wilson et al., 2011). Results presented here demonstrate that aphids also manipulate host physiology to promote the accumulation of an infestation-promoting factor in petiole exudates (Figure 2). This was in striking contrast with petiole exudates collected from uninfested Arabidopsis leaves, which contain an activity that adversely affected GPA population (Figure 2) (Louis et al., 2010a; Louis et al., 2010b). The infestation-promoting effect was significantly lower for petiole exudates collected from GPA-infested leaves of the lox5-1 mutant compared with the wild type (Figure 2), suggesting that LOX5 is required for the accumulation of this infestation-promoting activity in petiole exudates. In support of the involvement of LOX5 in promoting insect infestation, 9-HOD and 9-HPOD were found to enhance the size of the GPA population on an artificial diet (Figure 5B). Furthermore, 9-HOD and 9-HPOD accumulated at elevated levels in petiole exudates from GPA-infested leaves of wild-type Arabidopsis (Figure 5A). By comparison, the levels of these oxylipins were lower in petiole exudates collected from GPA-infested lox5-1 plants. Taken together, these results confirm that LOX5-synthesized oxylipins, or products thereof, promote GPA infestation.

As summarized in the model presented in Figure 7, our results indicate that Arabidopsis LOX5 provides a factor that facilitates aphid feeding from the sieve elements and thus sap consumption. In the absence of LOX5, GPAs spent less time in SEP (Table 1). The sugar-rich phloem sap consumed by aphids has an osmotic potential that is considerably more negative than the aphid hemolymph, and the resulting gradient could result in loss of water from the hemolymph, leading to dehydration of the insect (Douglas, 2006). One mechanism that aphids use to avoid dehydration is the ingestion of xylem sap (Powell and Hardie, 2002; Douglas, 2006). The diluted composition of xylem sap as compared with phloem sap (Mattson, 1980; Taiz and Zeiger, 2002) has been suggested to enable aphids to osmoregulate their hemolymph and prevent dehydration (Pompon et al., 2010; Pompon et al., 2011). The importance of xylem sap consumption to the insect is evident from studies with bird cherry-oat aphid (Rhopalosiphum padi). On wheat (Triticum aestivum) plants treated with the neonicotinoid insecticide thiamethoxam, which disrupts XP, the water content in bird cherry-oat aphids was reduced, and the size of the insect population was adversely affected (Daniels et al., 2009). Our results show that LOX5 also affects the ability of the insect to drink from the xylem. By comparison with the wild-type plant, when on the lox5 mutant, the insects did not exhibit XP, and water content in the insects was lower than in insects reared on wild-type plants (Table 1, Figure 3). Water content was restored in GPAs reared on 9-HOD-treated lox5 mutant plants (Figure 6B), confirming the importance of LOX5-derived oxylipin(s) in promoting hydration of GPA on Arabidopsis. At this stage, it is not clear whether the LOX5-dependent factor independently affects SEP and XP in GPA, or

![Figure 6](image)

**Figure 6.** LOX5-Derived 9-HPs Complement lox5-1 Phenotype.

(A) Complementation of the lox5-1 phenotype by 9-HOD applied to roots. No-choice test comparison of GPA numbers (mean ± SE; n = 6) on wild-type (WT) and lox5-1 plants that were irrigated with 9-HOD (10 and 100 nM). Controls were plants that did not receive 9-HOD (0 nM).

(B) Percentage water content in GPAs reared on lox5-1 plants treated with 9-HOD. Each bar represents the percentage content of water (mean ± SE; n = six groups of 30 to 40 GPAs) in GPAs reared on wild-type and lox5-1 mutant plants that were irrigated with 9-HOD (10 or 100 nM). Controls were wild-type and lox5-1 plants that did not receive 9-HOD (0 nM).

In (A) and (B), different letters above the bars indicate values that are significantly different (P < 0.05; GLM/ANOVA).

![Figure 7](image)

**Figure 7.** Model Depicting the Role of Root-Derived LOX5-Synthesized Oxylipin in Arabidopsis Interaction with GPAs.

GPA infestation on Arabidopsis shoots induces expression of LOX5 in roots within 3 h of infestation. The model suggests that a plant-derived and/or insect-derived signal originating in the shoot is delivered to the roots, leading to the stimulation of LOX5 expression and increased synthesis of 9-HPs. These 9-HPs are subsequently transported to the shoots, most likely through the vasculature, where they are utilized by GPAs as cues that facilitate both insect feeding from the sieve elements and water consumption from xylem and insect fecundity. Alternatively, these 9-HPs may counter and/or attenuate defenses targeting GPAs.
whether the reduced phloem sap consumption by insects reared on thelox5 mutant results in a lowered need for the insect to consume xylem sap.

Oxylipins synthesized by LOX5 may have a direct effect on GPA behavior and physiology, thus affecting time spent by the insect in SEP and XP and fecundity. 9-HOD is present in GPAs reared on plants, as compared with GPAs reared on an artificial diet (Harmel et al., 2007). Because GPAs lacks a 9-HOD synthesizing activity, it has been suggested that GPAs derive 9-HOD from the plant host (Harmel et al., 2007). Indeed, 9-HOD and other 9-LOX oxylipins are present in phloem sap (Harmel et al., 2007), and our results show that the content of 9-HOD and other 9-LOX oxylipins increases in petiole exudates collected from GPA-infested leaves compared with petiole exudates from uninfested leaves of Arabidopsis (Figure 5A). The fact that 9-HOD added to an artificial diet resulted in an increase in size of the insect population (Figure 5B) suggests that LOX5-synthesized oxylipins have the potential to directly affect GPA. The stimulation of insect feeding by LOX5-synthesized oxylipins and the resultant increase in nutrient consumption could be the cause of the larger GPA population on the wild type compared withlox5 mutant.

However, a more direct effect of 9-LOX products on insect fecundity cannot be ruled out. Because LOX5-synthesized oxylipins affect physiological processes in plants (Vellosillo et al., 2007; Vicente et al., 2011), it is also possible that the effect of LOX5-synthesized oxylipins on facilitating GPA infestation is mediated through their influence on molecular and physiological mechanisms in Arabidopsis. For example, 9-LOXs are known to influence activation of plant defense (Hwang and Hwang, 2010; López et al., 2011; Vicente et al., 2011). An antagonistic relationship between LOX5 oxylipins and Arabidopsis defense against GPAs could facilitate colonization of Arabidopsis by GPAs. It was recently shown that the 9-LOX oxylipin 9-keto-10(E),12(Z),15(Z)-octadecatrienoic acid modulates expression of JA- and salicylic acid-regulated genes (Vicente et al., 2011). Because JA signaling promotes defense against insects, and salicylic acid is known to antagonize the activation of JA signaling in plant–insect interaction (Walling, 2008), we monitored expression of the PATHOGENESIS-RELATED1 (PR1) and PDF1.2 genes, which are molecular markers for the activation of salicylic acid and JA signaling, respectively. As shown in Supplemental Figure 5 online, although expression of PR1 and PDF1.2 were elevated in GPA-infested plants, no discernible differences were observed in the temporal pattern and extent of expression of these genes between GPA-infested wild-type andlox5 mutant plants, suggesting that thelox5 phenotype is not associated with any obvious changes in the activation of salicylic acid and JA signaling. However, additional genetic studies are required to determine whether these and other hormones have a role in thelox5-mediated enhanced resistance to GPAs.

Grafting experiments confirmed that GPA performance on Arabidopsis foliage is influenced by the LOX5 genotype of roots (Figure 4B). Indeed, the level of LOX5-derived 9-HPs was elevated in roots of GPA-infested plants (Figure 4C), and application of 9-HOD to roots complemented thelox5-1 defect to restore fecundity and water content of GPAs (Figures 6A and 6B). Although the xylem is the potential route of transport of 9-LOXs from root to shoot, in the foliage, there is significant flow of xylem content into the phloem. Hence, whether these 9-LOX products are delivered to GPAs during XP or SEP is not clear and requires further investigation.

Expression of LOX5 was upregulated in roots of GPA-infested plants (Figure 4A), suggesting (as summarized in Figure 7) that an unknown factor travels from the GPA-infested foliage to stimulate LOX5 expression in the roots. Further research is necessary to identify the responsible signal(s). This shoot-to-root signal could be of plant origin, similar to the involvement of shoot-derived JA in nicotine production by roots of insect-infested Nicotiana sylvestris plants (Baldwin et al., 1994). It is equally possible that the shoot-to-root signal(s) that stimulates LOX5 expression in roots is derived from the aphid. Aphids inject saliva containing numerous proteins into the plant. Recent studies have indicated that salivary proteins are capable of eliciting responses in plants (De Vos and Jander, 2009; Bos et al., 2010). Furthermore, some salivary proteins have been shown to be required for efficient colonization of the host plant by the aphid (Mutti et al., 2008).

Although both LOX1 and LOX5 potentially synthesize the same oxylipins, our results (Figure 1A) indicate that only LOX5 had a discernible role in facilitating GPA infestation. This could be because of differences in the intracellular location of these two enzymes. Whereas LOX1 localizes to the chloroplast, LOX5 exhibits an extraplastidic pattern (see Supplemental Figure 1 online). This suggests that 9-HPs synthesized outside the chloroplast could have a different physiological and biological function than 9-HPs synthesized within the chloroplast. Although not much is known about the tissues in which LOX1 and LOX5 are expressed, any differences in these could also contribute to the differences in their involvement in facilitating GPA infestation.

In summary, evidence presented here indicates that roots are a critical source of oxylipins that promote colonization of the foliage by GPA. Although oxylipins are inducers of plant defense against pathogens and insects, and although volatile products of the LOX pathways are utilized by plants to attract predatory insects (Feussner and Wasternack, 2002), evidence presented here suggests that the GPA has adapted to cue in on specific 9-LOX–derived oxylipins for facilitating infestation. These results have broader ramifications to agriculture, because of the economic importance of the GPA as an insect pest of more than 50 families of plants (Blackman and Eastop, 2000) and a vector of several viral diseases in plants (Brault et al., 2010).

METHODS

Plant and Insect Cultivation

GPAs (Myzus persicae Sülzer) (Kansas State University, Museum of Entomological and Prairie Arthropod Research, voucher specimen 194) were reared on a 1:1 mixture of commercially available radish (Raphanus sativus) (Early Scarlet Globe) and mustard (Brassica juncea) (Florida Broadleaf) under a 14-h light (100 µE m−2 s−1) and 10-h dark regime at 22°C. For aphid hydration studies, adult aperuous (wingless) aphids were starved by confining them in a Petri dish for 4 to 6 h at 22°C. Arabidopsis thaliana accession Col-0 plants were cultivated under a 14-h light (100 µE m−2 s−1) and 10-h dark regime at 22°C in an autoclaved peat-based planting mixture (Premier Pro Mix-PGX; Premier Tech
Transgenic Plants
To generate 35S::LOX1 and 35S::LOX5 constructs in which the LOX1 and LOX5 coding regions are cloned downstream of the Cauliflower mosaic virus 35S promoter, RNA extracted from roots was used to amplify the coding region of LOX1 and LOX5 using the following primer combinations: LOX1-C-F and LOX1-C-R (full length) and LOX5-C-F and LOX5-C-R (full length) (see Supplemental Table 1 online). The PCR products were cloned into the pCR8/GW/TOPO vector (Invitrogen) and subsequently used in a LR recombination reaction with the destination vectors pMDC32 (Curts and Grossniklaus, 2003) to yield pMDC32-35S::LOX1 and pMDC32-35S::LOX5 plasmids. To generate 35S::LOX1-GFP and 35S::LOX5-GFP constructs in which the GFP coding region is fused to the C terminus (last amino acid) of LOX1 and LOX5, RNA extracted from roots was used to amplify the coding region of LOX1 and LOX5 using the following primer combinations: LOX1-C-F and LOX1-NS-R (full length without stop codon) and LOX5-C-F and LOX5-NS-R (full length without stop codon) (see Supplemental Table 1 online). The PCR products were cloned into the pCR8/GW/TOPO vector (Invitrogen) and subsequently used in a LR recombination reaction with the destination vectors pMDC32 (Curts and Grossniklaus, 2003) to yield pMDC32-35S::LOX1-GFP and pMDC32-35S::LOX5-GFP plasmids. The pMDC32-35S::LOX1, pMDC32-35S::LOX5, pMDC32-35S::LOX1-GFP, and pMDC32-35S::LOX5-GFP plasmids were electroporated into Agrobacterium tumefaciens strain GV3101 and used to transform Arabidopsis by the floral-dip method (Clough and Bent, 1998). Transgenic plants were selected for their resistance to hygromycin. To generate 35S::LOX1 and 35S::LOX1-GFP plants, the pMDC32-35S::LOX1 and pMDC32-35S::LOX1-GFP constructs, respectively, were transformed into the lox1-1 mutant, whereas the pMDC32-35S::LOX5 and pMDC32-35S::LOX5-GFP constructs were transformed into the lox5-1 and lox5-3 mutants to generate transgenic 35S::LOX5 and 35S::LOX5-GFP plants, respectively.

No-Choice Assay
For no-choice assays, 20 asexual adult aphids were released on each plant, and the total numbers of insects (adults + nymphs) were counted 2 d later (Pegadaraju et al., 2005).

EPG Analysis
The EPG (Walker, 2000) technique was used to monitor GPA feeding behavior as previously described (Pegadaraju et al., 2007). A total of 16 replicates were conducted each with a different pair of GPA and plant. Data obtained from EPG were analyzed by nonparametric Kruskal-Wallis test (Minitab v15).

Petiole Exudate Collection
Petiole exudates were collected from Arabidopsis leaves using a previously described method (Chaturvedi et al., 2008) with a few modifications. Leaves were cut at the base of their petioles, and the petiole was immediately dipped in 50% ethanol, followed by 0.0005% bleach, and then was immersed in 1 mM EDTA (pH 8.0) solution. After 20 min, the base of the petiole was cut, and the petiole end was immediately placed in a fresh solution (3 mL) of 1 mM EDTA (pH 8.0) contained in a well of a 24-well tissue culture plate (ICN Biochemical; http://www.mpbio.com), six leaves to each well. High humidity was maintained by placing the entire set-up on wet paper towels under a transparent dome. The leaves were allowed to exude for a period of 12 h. After the exudation period, the contents of each well were filtered through a 0.45-μm syringe filter (Fisherbrand 25-mm Syringe Filter; http://www.fishersci.com). Petiole exudates from six wells were pooled, lyophilized, and resuspended in 2 mL of 1 mM EDTA (pH 8.0) for use in all experiments. Petiole exudates were collected from unin-fest (+GPA) and GPA-infested (+GPA) plants, 48 h after infestation. For collection of petiole exudates from GPA-infested plants, only leaves that had a minimum of five aphids were used.

Artificial Diet Assays
An artificial diet (Mittler et al., 1970) was used for all feeding trial bioassays. A feeding chamber was constructed as previously described (Louis et al., 2010a). Each feeding chamber contained a total volume of 500 μL, which included the artificial diet with or without petiole exudates or chemicals. Six adult asexual GPA were placed on each feeding chamber for 2 d, at the end of which the total number of aphids (adults + nymphs) in each chamber was determined.

Measurement of GPA Body Water Content
Aphids were starved for 14 to 16 h by placing them in a Petri dish without water. Twenty starved aphids were allowed to feed on each Arabidopsis plant for 12 h. To estimate water content, 30 to 40 adult aphids from two plants were collected and immediately weighed. Dry weights were obtained after drying the aphids at 60°C for 24 h. The water content of aphids was determined by subtracting the dry weight from the fresh weight of the aphids.

Micrografting
Single-hypocotyl grafts were constructed as previously described (Turnbull et al., 2002). Arabidopsis seedlings were germinated on one-half-strength Murashige and Skoog plates with 2% agar and 1% Suc under a 14-h light (100 μE·m⁻²·s⁻¹)/10-h dark regime at 22°C. One d before grafting, the 10-d-old seedlings were moved to 27°C. The scion and rootstock were cut transversely and aligned precisely under sterile conditions without a grafting collar. The grafted seedlings were placed back at 27°C for 3 d to 4 d to promote graft union and were monitored every day, and any adventitious roots that were observed were crushed with forceps. After 7 to 12 d, grafts that were functional were transplanted to soil. No-choice assays were performed on plants that were 4 to 5 weeks old. Genotype of the rootstock and scion for each graft was confirmed by PCR performed on DNA extracted from root and shoot for each set of plants, respectively.

Confocal Laser Scanning Microscopy
Protoplasts from leaves of the wild-type, 35S::GFP, 35S::LOX1-GFP, and 35S::LOX5-GFP plants were isolated as previously reported (Jin et al., 2001) and observed with a Zeiss 200M inverted optical microscope fitted with a CSU-10 Yokogawa Confocal scanner (McBain Instruments) and captured with a digital camera (Hamamatsu). Excitation wavelengths and emission filters were 488 nm/bandpass 505 to 535 nm for GFP and 488 nm/bandpass 672 to 712 nm for chloroplast autofluorescence. Three-dimensional images were obtained using Imaris 6.2 (BitPlane) software and are presented as stacks of neighboring sections.

Genomic PCR
All primers used in this study are listed in Supplemental Table 1 online. Plants homozygous for lox1-1 (SALK_059431), lox1-3 (SALK_012188),...
performed under the following conditions: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 2 min, with a final extension of 72°C for 5 min.

**RT-PCR and Quantitative Real-Time PCR**

RNA extraction from leaves and roots and cDNA synthesis was performed as previously described (Pegadaraju et al., 2005). DNA contamination in samples was removed by treatment with RNase-free DNase (Sigma-Aldrich). For each time point and treatment, RNA was collected from three biological replicates, each consisting of leaves pooled from four plants. Gene-specific primers used for RT-PCR and quantitative real-time PCR are listed in Supplemental Table 1 online and were identified using the AFRTPRimer database (http://atrpprimer.kaist.ac.kr/) (Han and Kim, 2006). The PCR conditions for all RT-PCR reactions were as follows: 95°C for 5 min, followed by 25 cycles of 95°C for 30 s, 58°C for 45 s, and 72°C for 45 s, followed by a final extension step of 72°C for 5 min. Real-time PCR was performed with Sybr Green PCR Master Mix (Applied Biosystems) on an Eco qPCR system (iLumina) using the following amplification protocol: 10 min polymerase activation and denaturation at 95°C, 40 cycles of 95°C for 10 s, 58°C for 30 s, and 72°C for 30 s. This was followed by a product melt to confirm a single PCR product. The level of LOX5 expression was normalized to that of EF1α (At5g60390) by subtracting the cycle threshold value of EF1α from the cycle threshold value of LOX5.

**Oxylipin Profiling**

Lipids were extracted from plant samples as previously described (Göbel et al., 2002). The extracted lipids were resuspended in 100 µL of methanol:water (80:20 v/v). Oxylipins in the lipid extract were first purified by reverse-phase HPLC using an Agilent 1100 HPLC coupled to a UV diode array detector. A Nucleosil 120-5 C18 column (4.6 × 150 mm, 5 µm; Macherey-Nagel) with a binary gradient solvent system [solvent A: methanol:water:acetic acid (80:20:1 v/v/v) and solvent B: methanol:acetic acid (100:0.1 v/v)] and a flow rate of 0.125 mL/min were used. A strong abundance of LOX5 was followed by a product melt to confirm a single PCR product. The level of expression was normalized to that of EF1α (At5g60390) by subtracting the cycle threshold value of EF1α from the cycle threshold value of LOX5.

**Statistical Analysis**

For all assays, t test or analysis of means using the general linear model (GLM)/Analysis of Variance (ANOVA) was used to separate the means. For the EPG analysis, the mean time spent by the aphids on various activities was analyzed using the nonparametric Kruskal-Wallis test (Minitab v15).

**Accession Numbers**

Sequence data from this article can be found in the Arabidopsis Genome Initiative and GenBank/EMBL data libraries under accession numbers LOX1 (At1g55020), LOX5 (At3g22400), and EF1α (At5g60390).

---

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure 1.** LOX1 and LOX5 Are Localized to Different Subcellular Compartments.

**Supplemental Figure 2.** 9-LOX Mutants of Arabidopsis.

**Supplemental Figure 3.** LOX5 Expression in Arabidopsis Leaves.

**Supplemental Figure 4.** Verification of Graft Integrity.

**Supplemental Figure 5.** PR1 and PDF1.2 Expression in Wild-Type andlox5-1Mutant Plants.

**Supplemental Table 1.** Primers Used in This Study.

---

**ACKNOWLEDGMENTS**

We thank Lon Turnbull for assistance with fluorescence confocal microscopy, Joe Louis for EPG setup, and Kent D. Chapman for access to analytical instrumentation, which was purchased with funds from the U.S. Department of Energy Office of Science, Basic Energy Sciences Program (contract DE-FG02-05ER15647) and used for oxylipin profiling. This work was supported by grants from the National Science Foundation (Division of Molecular and Cellular Biosciences-0920600) and Division of Integrative Organismal Systems-0919192) to Jyoti Shah and a graduate fellowship awarded to Vamsi Nalam by the College of Arts and Science at the University of North Texas.

**AUTHOR CONTRIBUTIONS**

V.J.N. and J.S. designed the research; V.J.N., J.K., and S.S. performed the research; V.J.N., J.K., and J.S. analyzed data; and V.J.N. and J.S. wrote the article.

Received November 22, 2011; revised January 30, 2012; accepted March 16, 2012; published April 3, 2012.

**REFERENCES**


Gao, X., Starr, J., Leptinotarsa decemlineata (Sulzer) or a chewing insect (Leptinotarsa decemlineata Say) on potato plants (Solanum tuberosum L.) induce differential changes in volatile compounds released and oxylipin synthesis. J. Exp. Bot. 60: 1231–1240.


**Root-Derived Oxylipins Promote Green Peach Aphid Performance on Arabidopsis Foliage**

Vamsi J. Nalam, Jantana Keeretaweep, Sujon Sarowar and Jyoti Shah

*Plant Cell*; originally published online April 3, 2012; DOI 10.1105/tpc.111.094110

This information is current as of July 1, 2017

<table>
<thead>
<tr>
<th>Supplemental Data</th>
<th>/content/suppl/2012/03/20/tpc.111.094110.DC1.html</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>/content/suppl/2012/04/06/tpc.111.094110.DC2.html</td>
</tr>
<tr>
<td>eTOCs</td>
<td>Sign up for eTOCs at: <a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a></td>
</tr>
<tr>
<td>CiteTrack Alerts</td>
<td>Sign up for CiteTrack Alerts at: <a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a></td>
</tr>
<tr>
<td>Subscription Information</td>
<td>Subscription Information for <em>The Plant Cell</em> and <em>Plant Physiology</em> is available at: <a href="http://www.aspb.org/publications/subscriptions.cfm">http://www.aspb.org/publications/subscriptions.cfm</a></td>
</tr>
</tbody>
</table>