



## Plants Under Attack

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To cite this article: Martin De Vos, Vivian R. Van Oosten, Georg Jander, Marcel Dicke & Corné M.J. Pieterse (2007) Plants Under Attack, *Plant Signaling & Behavior*, 2:6, 527-529, DOI: [10.4161/psb.2.6.4663](https://doi.org/10.4161/psb.2.6.4663)

To link to this article: <https://doi.org/10.4161/psb.2.6.4663>



Published online: 01 Nov 2007.



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## Article Addendum

# Plants Under Attack

## Multiple Interactions with Insects and Microbes

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Original manuscript submitted: 06/29/07

Manuscript accepted: 07/05/07

Previously published online as a *Plant Signaling & Behavior* E-publication: <http://www.landesbioscience.com/journals/psb/article/4663>

### KEY WORDS

Arabidopsis, induced resistance, defense signaling, *Myzus persicae*

### Addendum to:

#### *Herbivore-Induced Resistance Against Microbial Pathogens in Arabidopsis*

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Dicke M, Van Loon LC, Pieterse CM

Plant Physiol 2006; 142:352–63

PMID: 16829584

DOI: 10.1104/pp.106.083907

### ABSTRACT

To defend themselves, plants activate inducible defense mechanisms that are effective against the invader that is encountered. There is partial overlap in the defense signaling pathways that are induced by insect herbivores and microbial pathogens that may result in cross-resistance. We have previously shown that infestation by tissue-chewing *Pieris rapae* larvae induces resistance in *Arabidopsis thaliana* against subsequent attack by the microbial pathogens *Pseudomonas syringae* pv. *tomato* (*Pst*), *Xanthomonas campestris* pv. *armoraciae* (*Xca*) and turnip crinkle virus (TCV). Phloem-feeding aphids, such as the generalist *Myzus persicae*, have a stealthy feeding strategy that is very different from chewing by lepidopteran larvae. Yet, *M. persicae* feeding results in a large transcriptomic change. Here, we report on the effectiveness of the defense response that is triggered by *M. persicae* infestation, as well as the sensitivity of *M. persicae* to microbially-induced resistance. *M. persicae* reproduction was not affected by prior conspecific feeding, nor was aphid-induced resistance effective against subsequent attack by *Pst*, *Xca* or TCV. Moreover, induced systemic resistance (ISR) triggered by beneficial *Pseudomonas fluorescens* rhizobacteria was not effective against *M. persicae*. However, systemic acquired resistance (SAR) induced by prior infection with avirulent *Pst* was associated with reduced aphid reproduction. These data provide insight into the effectiveness of pathogen and insect resistance and highlight the complexity of the defense responses that are triggered during multitrophic plant-attacker interactions.

Plants are abundantly present on earth and are at the basis of almost all food webs. Plants face a multitude of attackers such as herbivorous insects and pathogenic microbes. While there are ca. 300,000 plant species, there are expected to be ca. Three million species of herbivorous insects.<sup>1</sup> The diversity of pathogenic microbes is less well characterized but their threat to plants is equally renowned.<sup>2</sup> To effectively combat invasion by pathogens and insects plants have evolved sophisticated strategies to “perceive” biotic interactions and to translate this “perception” into an appropriate defensive or conducive response.<sup>3–6</sup> Recent genomics research revealed that the plant’s capacity to respond to the enormous diversity of parasites and beneficials is highly flexible.<sup>7–12</sup> Signaling networks that are recruited by the plant in response to pathogens and insects overlap, indicating that the regulation of the plant’s adaptive response is finely-balanced between protection against microbial and insect aggressors.

To understand how plants integrate pathogen- and insect-induced signals into specific defense responses, we previously monitored the dynamic production of the defense signals salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), and performed large-scale gene expression studies in *Arabidopsis thaliana* upon attack by a set of microbial pathogens and herbivorous insects with different modes of attack.<sup>7</sup> The results obtained in this study indicated that plants induce a highly attacker-specific gene expression pattern that is shaped, among other signals, by SA, JA and ET. Next, we investigated whether insect-induced resistance provides cross-resistance against pathogens in *Arabidopsis*. We demonstrated that resistance induced by larvae of the cabbage white butterfly *Pieris rapae* is not only effective against *P. rapae* itself, but also against several microbial pathogens.<sup>13</sup> For instance, *P. rapae* feeding locally reduced symptoms caused by the bacterial pathogens *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Xanthomonas campestris* pv. *armoraciae* (*Xca*). Moreover, *P. rapae* feeding induced local and systemic resistance against turnip crinkle virus (TCV).

In contrast to tissue maceration by larvae of *P. rapae*, aphids minimize damage to their hosts. They use their flexible stylets to probe intercellularly between the plant cells to reach the phloem and feed for a prolonged period of time from a single sieve element.<sup>14</sup> These different feeding strategies result in very distinct gene expression patterns upon attack

by *Myzus persicae* (green peach aphid) or *P. rapae*.<sup>7</sup> Here, we investigated the spectrum of effectiveness of aphid-induced resistance against several microbial pathogens, including *Pst*, *Xca* and TCV. In addition, we studied microbially-induced resistance against *M. persicae*.

Previously, we demonstrated that three days of feeding by 40 aphids does not result in detectable increases in the levels of SA, JA or ET.<sup>7</sup> Surprisingly, gene expression profiling of Arabidopsis after 48 and 72 hour of *M. persicae* infestation revealed that aphids induce a major reprogramming of the Arabidopsis transcriptome.<sup>7</sup> The majority of the genes with changed expression are predicted to play a role in plant secondary metabolism. Figure 1A shows that aphid feeding resulted in a ~40% reduction in plant fresh weight, presumably through removal of photoassimilates (carbon) and amino acids (nitrogen) from the phloem. Hence, the previously observed changes in the transcriptome may be caused by a shift in source-sink relations.

In order to investigate whether the response that is triggered in Arabidopsis upon feeding by *M. persicae* results in enhanced aphid resistance, we monitored the reproduction of this attacker. Arabidopsis accession Columbia-0 (Col-0) plants were infested with 20 aphids. After 72 hours, all aphids were removed and plants were infested with a single adult aphid. *M. persicae* reproduction was the same on pre-treated and control plants (Fig. 1B), indicating that Arabidopsis does not recognize the attacker or cannot mount an effective defense response against this attacker. The latter is more likely, as Arabidopsis plants showed large transcriptional changes upon *M. persicae* infestation. Also in potato, *M. persicae* feeding did not result in induced resistance to *M. persicae*.<sup>15</sup> It is thought that aphids manipulate plant responses and thereby avoid or suppress induction of effective defense responses.<sup>16,17</sup>

Similar experiments were performed with the challenging pathogens *Pst*, *Xca* and TCV. Although the overlap in genes differentially expressed upon *M. persicae* and *Pst* attack was approximately 30%,<sup>7</sup> prior feeding by aphids did not cause cross-resistance to either *Pst* or *Xca* (Fig. 1C). Green peach aphids are capable of transmitting over 100 virus species,<sup>18</sup> including TCV. Hence, aphid feeding could serve as a cue for the plant to prepare for potential secondary viral infections. We hypothesized that prior feeding by *M. persicae* would lead to induced resistance against TCV. In Col-0 plants, TCV spreads systemically throughout the plant and does not produce visible disease symptoms. Accession Dijon-0 (Di-0) exhibits a hypersensitive response (HR) to TCV, resulting in confined necrotic lesions.<sup>19-21</sup>

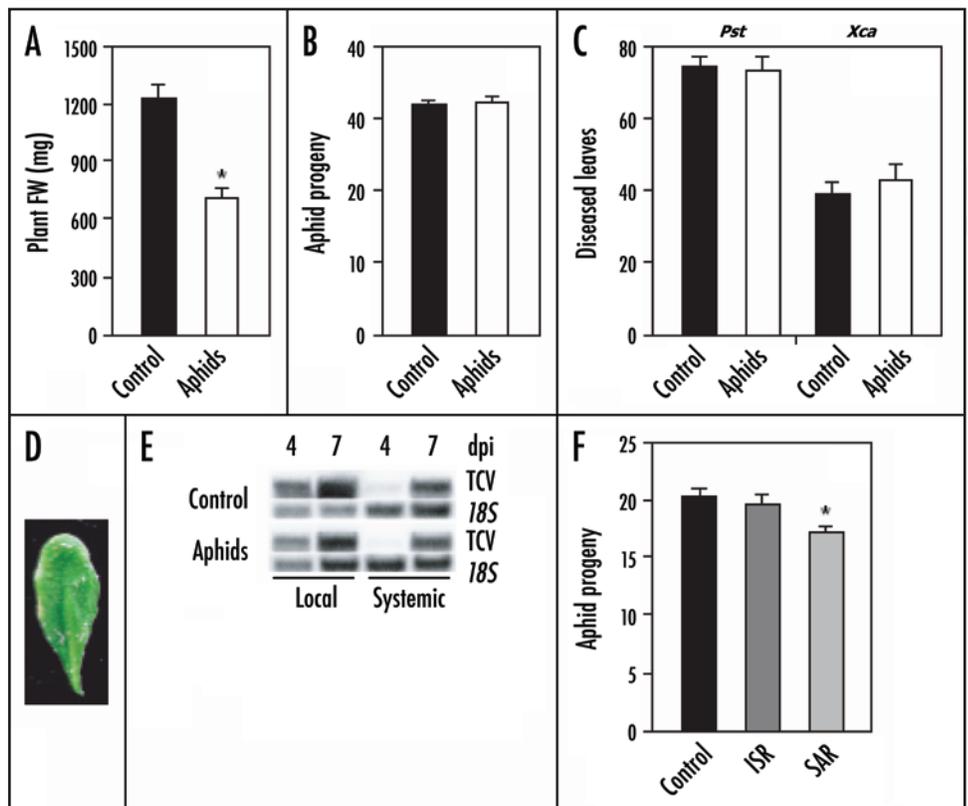


Figure 1. *Arabidopsis-Myzus persicae* interactions. (A) Infestation of five-week-old Arabidopsis Col-0 plants with 20 adult aphids for three days significantly reduced the fresh weight (FW) of Arabidopsis rosettes ( $n = 20$ ). (B) Progeny per adult aphid while feeding for seven days on un-induced Col-0 plants and on plants that had been pre-infested with aphids for three days ( $n = 15$ ). (C) Percentage of leaves with disease symptoms caused by the bacterial pathogens *P. syringae* pv. *tomato* DC3000 (*Pst*) or *X. campestris* pv. *armoraciae* (*Xca*) in control and aphid-induced Col-0 plants ( $n = 15$ ). (D) Aphid-vectored TCV symptoms on Arabidopsis Di-0 plants. (E) Local and systemic accumulation of TCV RNA at four and seven days post inoculation (dpi) in inoculated control and aphid-induced Col-0 plants. (F) The number of offspring produced per adult aphid while feeding for six days on uninoculated Col-0 plants and on plants expressing *P. fluorescens* WCS417r-mediated ISR, or *P. syringae* pv. *tomato* DC3000(*avrRpt2*)-induced SAR ( $n = 20$ ). Green peach aphids (*Myzus persicae*) were reared on radish (*Raphanus sativus*), in a greenhouse (25°C, RH 50–70%, L16:D8h). The 16 h light period prevented sexual reproduction, keeping the population clonal. A synchronous colony was started by transferring apterous adults to uninfested radish plants. The aphids were enclosed in clip-cages. After 24 h, the adults were removed and the newly born nymphs kept until they moulted. Using a fine paint brush, single, recently moulted (one to three days) adult apterae were transferred to single Col-0 plants. Each plant was confined in a Magenta GA-7 vessel. Offspring per aphid was determined after six to seven days in a climate chamber (23°C, RH 70%, L16:D8h). For induction treatments, all aphids were removed after three days, after which the plants were challenged with fresh aphids or pathogens. Induction of ISR and SAR, and pathogen assays were performed as described (refs.13,25). Student's *t*-test (A–C) or one-way ANOVA with a Bonferroni post-hoc test (F) ( $p < 0.05$ ) was performed to test for significant differences. Statistically significant differences compared to the control are indicated with asterisks.

First, we tested if *M. persicae* is able to transmit TCV to non-infected tissue in Arabidopsis Di-0. Indeed, as a result of *M. persicae* feeding, TCV was vectored to uninfected leaves of Arabidopsis Di-0 plants where they cause HR-like symptoms (Fig. 1D). Next, *M. persicae*-induced resistance against TCV was tested in Col-0 by monitoring the amount of TCV RNA in local and systemic leaves at different time points after infection. Control and aphid-induced plants showed equal amounts of TCV RNA (Fig. 1E), indicating that the responses triggered in Arabidopsis by aphid feeding did not result in cross-resistance against TCV. Together, these results indicate that infestation of Arabidopsis by *M. persicae* does not result in enhanced resistance to the microbial pathogens *Pst*, *Xca* and TCV.

In reciprocal experiments, aphid reproduction was assessed after elicitation of microbially-induced resistance. Rhizobacteria-mediated induced systemic resistance (ISR),<sup>22</sup> triggered by *Pseudomonas fluorescens* WCS417r,<sup>23</sup> was not effective against *M. persicae* (Fig. 1F). However, systemic acquired resistance (SAR),<sup>24</sup> triggered by infection with avirulent *Pst*,<sup>25</sup> significantly reduced aphid reproduction. The number of aphid offspring on SAR-expressing plants was significantly reduced in comparison to control plants (Fig. 1F). Although it is unclear what signals contribute to this effective aphid control, it is likely that a rise in SA levels, which is typically associated with SAR, plays a dominant role in aphid resistance.<sup>26</sup> Recently, it was shown in *Arabidopsis* that nymphs of the phloem-feeding insect *Bemisia tabaci* (Silverleaf Whitefly) sabotage effectual JA-dependent host defenses by activating the antagonistic SA signaling pathway.<sup>27</sup> Since *M. persicae* has a similar feeding strategy, and the SA marker gene *PR-1* is activated upon aphid feeding,<sup>7</sup> the inhibitory effect of pathogen-induced SAR on aphid development may resemble this SA-mediated antagonistic effect. On the other hand, JA has also been implicated in resistance to *M. persicae*,<sup>28</sup> and since JA is systemically produced upon elicitation of SAR by avirulent *Pst*,<sup>29,30</sup> we can not exclude a role for JA in defense against *M. persicae*. These data show that a biotic interaction between a plant and one of its attackers may influence interactions with subsequent attackers.

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