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Green peach aphid infestation induces Arabidopsis *PHYTOALEXIN-DEFICIENT4* expression at site of insect feeding

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The *Arabidopsis thaliana* PHYTOALEXIN-DEFICIENT4 (PAD4) protein, which has homology to lipases, is required for phloem-based resistance against the green peach aphid (GPA; *Myzus persicae* Sülzer). PAD4 modulates antibiotic and antixenotic defenses against GPA. PAD4 in conjunction with its interacting partner ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) also functions in basal resistance to bacterial and oomycete pathogens by promoting salicylic acid (SA)-dependent and SA-independent defenses. By contrast, neither EDS1 nor SA is required for PAD4-controlled defense against GPA. Distinct molecular activities of PAD4 are involved in different aspects of Arabidopsis defense against GPA and pathogens. Histochemical analysis of plants containing a *PAD4*$_p$:*GUS* chimera, which expresses the GUS reporter from the *PAD4* promoter, indicated strong *PAD4* promoter activity at the site of penetration of the vasculature by the insect stylet. GUS activity was also observed in non-vascular tissues of GPA-infested leaves, thus raising the possibility that a combination of distinct PAD4 activities in vascular and non-vascular tissues contribute to Arabidopsis defense against GPA.

Aphids (Hemiptera: Aphididae) are important pests of plants that use their slender stylets to feed on phloem sap.[1] The penetration path of aphid stylet is predominantly intercellular. However, occasionally, the aphid stylet pierces host cells. The green peach aphid (GPA), *Myzus persicae* (Sülzer), is one of the most damaging pests of many crop plants.[2] It has a host range covering more than hundred families of plants.[1] In addition, GPA is an important vector for more than hundred economically important plant viruses.[3] The interaction between *Arabidopsis thaliana* and GPA has provided an excellent model system to study the molecular mechanism of plant defense against aphids.[4] We have previously shown that expression of the Arabidopsis *PHYTOALEXIN-DEFICIENT4* (*PAD4*) gene, which encodes a protein with homology to acyl hydrolases/lipases, is induced in response to GPA infestation.[5] *PAD4* is an important modulator of antibiotic defenses that adversely impact GPA fecundity, and antixenotic defenses that impact plant choice by insect and feeding behavior.[5-8] Electrical monitoring of insect feeding behavior confirmed a vital role for *PAD4* in providing phloem-based resistance against GPA.[6] Recently, we established that serine at position 118 (S118) in PAD4, which corresponds to a key active site residue in other eukaryotic lipases, is critical for limiting GPA feeding from the sieve elements and for controlling aphid fecundity.[8] However, S118 was dispensable for deterring insect settling on plants.[8]

The PAD4 Interacting Partner ENHANCED DISEASE SUSCEPTIBILITY1 is Not Required for Providing Antixenotic Defenses Against GPA

In Arabidopsis, the PAD4 protein along with its interacting partner, EDS1 (ENHANCED DISEASE SUSCEPTIBILITY1), which like PAD4 contains a region with homology to active site of acyl hydrolases/lipases, are also required for promoting defense against bacterial and oomycete pathogens.[9-11] *EDS1* is required for accumulation of PAD4 protein in pathogen-infected tissues.[10] The PAD4-EDS1 activity promotes accumulation of the defense signaling molecule salicylic acid (SA) in pathogen-infected plants. Like *PAD4*, *EDS1* expression is also induced in response to GPA infestation.[6,8] However, PAD4's involvement in Arabidopsis defense against GPA is molecularly distinct from its involvement in defense against pathogens.[8] By contrast to the larger population size of GPA on the *pad4* mutant, aphid numbers were comparably low on the *eds1* mutant and wild-type (WT) plants, thus indicating that *EDS1* is not critical for the *PAD4*-dependent mechanism that controls GPA population size on Arabidopsis.[6] Furthermore, we have

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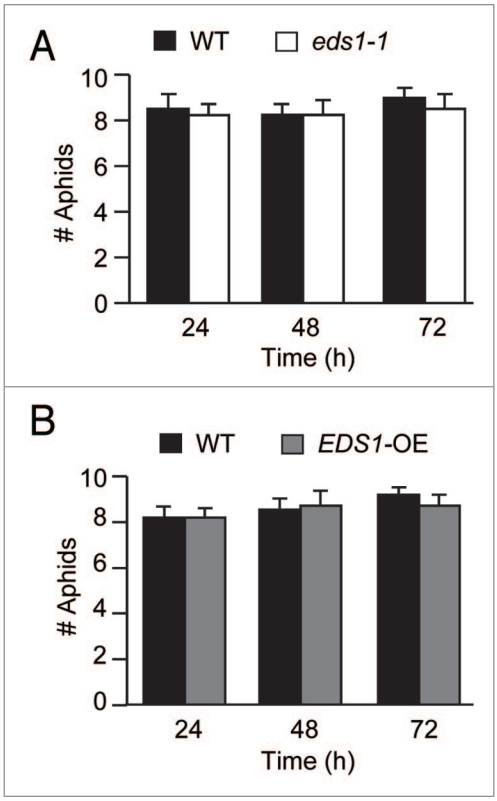

Figure 1. *EDS1* is not required for plant choice by insect. Choice assay: Twenty adult apterous (wingless) aphids were released at the center of each pot containing one WT accession Wassilewskija (Ws-0) and one *eds1-1* plant **(A),** or one WT Ws-0 and one *EDS1*-OE plant **(B)**, equidistant from the two plants. The numbers of released insects that had settled on plants of each genotype was determined at 24, 48 and 72 h post release. Values are the average of aphid counts on a minimum of eight plants of each genotype for each time point. Error bars represent SE. The means were separated using pooled χ^2 test. There were no significant difference between insect numbers on the WT and the *eds1-1*, or WT and the *EDS1*-OE plants. The experiments in A and B were independently conducted two times.

shown that redundancy between *EDS1* and *SID2* (*SALICYLIC ACID INDUCTION DEFICIENT2*), which is involved in the synthesis of SA, is not critical for controlling GPA population size on Arabidopsis. To determine if *EDS1* has a role in antixenosis that impacts plant choice by GPA, insects were given a choice between the WT and the *eds1* mutant plants. As shown in **Figure 1A**, the average number of GPAs that had settled on the WT was comparable to that on the *eds1* mutant. Similarly, the insects showed no preference for the WT over the *EDS1*-OE transgenic plant in which *EDS1* was constitutively overexpressed from the *Cauliflower mosaic virus 35S* gene promoter (**Fig. 1B**).[8] These results further support our suggestion that PAD4 functions independent of EDS1, in mediating both antibiotic and antixenotic defenses against GPA.

PAD4 Promoter is Activated at Site of Penetration of Vascular Tissue by the Insect Stylet

PAD4 activity is likely required in the vasculature to control GPA infestation. Alternatively, although not exclusively, PAD4 activity in another tissue type(s) may contribute to basal resistance against GPA. To determine if *PAD4* expression is induced in the vascular tissues in response to GPA infestation, we generated transgenic Arabidopsis plants (accession Columbia) containing a chimeric reporter consisting of approximately 1.6 Kb of the *PAD4* promoter (*PAD4*$_p$) cloned upstream of the bacterial β-glucuronidase (GUS) coding sequence. GUS activity was monitored in situ with the synthetic substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) in un-infested and GPA-infested leaves of *PAD4*$_p$:*GUS* plants. As shown in **Figure 2A**, by comparison to the un-infested leaves, which showed negligible GUS activity, blue coloration indicative of GUS activity was observed at several places around the vasculature in GPA-infested leaves. Strongest staining was observed at sites of penetration of vascular tissue by the insect stylet (**Fig. 2B**). These results are supportive of our suggestion that PAD4 functions in the vascular tissues to control insect infestation.[6,7,12] Additional experiments are required to determine if expression of *PAD4* in the vascular tissues is sufficient to complement one or more *pad4* mutant defects in plant defense. Since GUS activity was also observed in clusters of non-vascular cells (**Fig. 2A**), it is likely that PAD4 function in defense against GPA is required in other tissue types, as well. Our recent studies with a missense version of PAD4 in which serine at position 118 was replaced with alanine, suggested that distinct molecular activities of PAD4 contribute to different aspects of defense against GPA. Whether these distinct PAD4 activities are manifested in different tissue types, as alluded by the expression pattern of *PAD4*$_p$:*GUS* in GPA-infested leaves (**Fig. 2A and B**), remains to be determined.

Disclosure of Potential Conflict of Interest

The authors have no potential conflicts of interest.

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Note

GenBank IDs: *PAD4* (At3g52430), *EDS1* (At3g48090).

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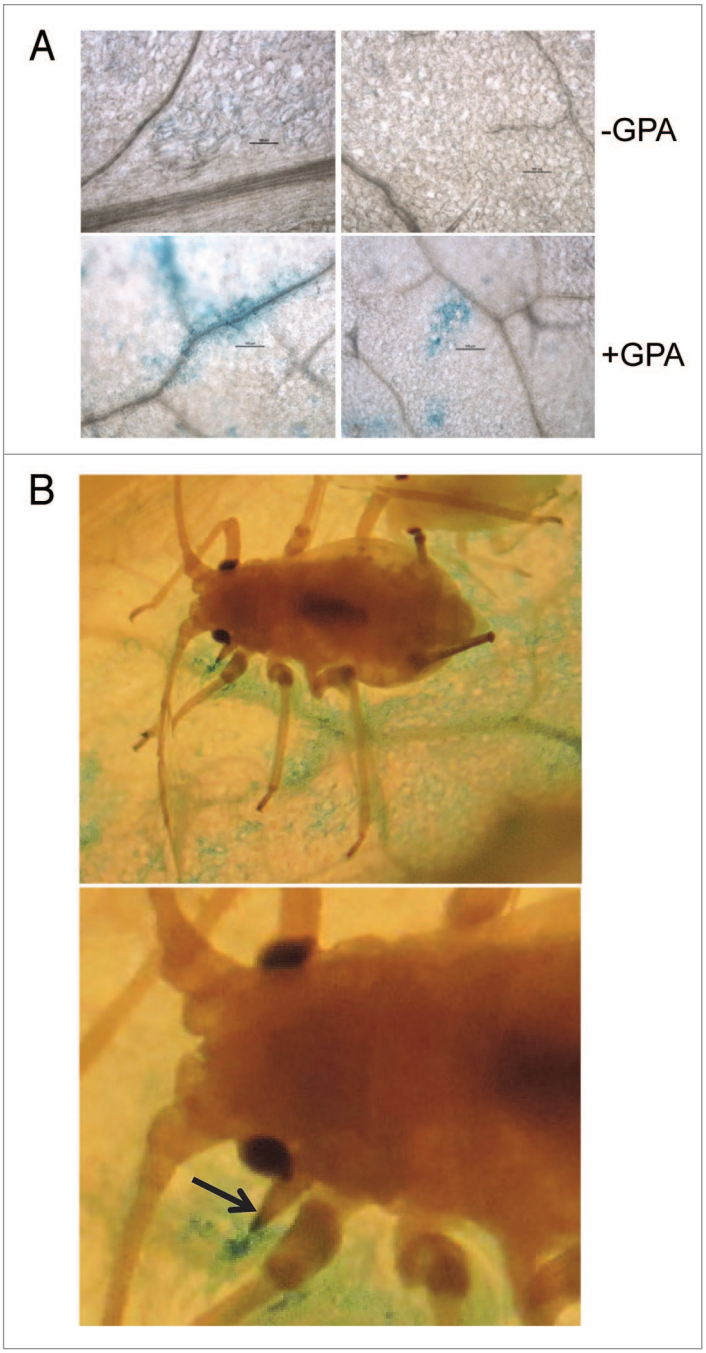

Figure 2. *PAD4*$_p$:*GUS* expression in un-infested and GPA-infested leaves. (**A**) Histochemical staining for GUS activity in un-infested (-GPA) (two upper panels) and GPA-infested (+GPA) (two lower panels) leaves, 48 h post release of insects. Left lower panel shows GUS activity around vasculature, and right lower panel shows GUS activity in cells distinct from vascular tissues. (**B**) Histochemical staining for GUS activity around vascular tissue near an insect (upper panel). Lower panel shows a close up view of GUS activity at site of penetration of plant tissue by the insect stylet. The aphid rostrum, the appendage to which the stylet is attached, is indicated by a black arrow.