University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Faculty Publications: Department of Entomology | Entomology, Department of

11-2014

Plant defense against aphids: The PAD4 signalling nexus

Joe Louis
University of Nebraska–Lincoln, joelouis@unl.edu

Jyoti Shah
University of Nebraska–Lincoln

Follow this and additional works at: http://digitalcommons.unl.edu/entomologyfacpub

Louis, Joe and Shah, Jyoti, "Plant defense against aphids: The PAD4 signalling nexus" (2014). *Faculty Publications: Department of Entomology.* Paper 367.
http://digitalcommons.unl.edu/entomologyfacpub/367

This Article is brought to you for free and open access by the Entomology, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications: Department of Entomology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Published in *Journal of Experimental Botany* (2014); doi: 10.1093/jxb/eru454
Copyright © 2014 Joe Louis and Jyoti Shah. Published by Oxford University Press on behalf of the Society for Experimental Biology. Used by permission.
Published online November 21, 2014.

UNIVERSITY OF Nebraska Lincoln
digitalcommons.unl.edu

Plant defense against aphids: The PAD4 signalling nexus

Joe Louis[1] and Jyoti Shah[2]

1. Department of Entomology and Department of Biochemistry, University of Nebraska–Lincoln, Lincoln, NE 68583
2. Department of Biological Sciences, University of North Texas, Denton, TX 76203

Corresponding author — J. Louis, email joelouis@unl.edu

Abstract
In *Arabidopsis thaliana*, *PHYTOALEXIN DEFICIENT 4* (*PAD4*) functions as a key player in modulating defense against the phloem sap-feeding aphid *Myzus persicae* (Sülzer), more commonly known as the green peach aphid (GPA), an important pest of a wide variety of plants. *PAD4* controls antibiosis and antixenosis against the GPA. In addition, *PAD4* deters aphid feeding from sieve elements on *Arabidopsis*. In the past few years, substantial progress has been made in dissecting the role of *PAD4* and its interaction with other signalling components in limiting aphid infestation. Several key genes/mechanisms involved in providing aphid resistance/susceptibility in *Arabidopsis* regulate the aphid infestation-stimulated expression of *PAD4*. Together, *PAD4* and its interacting signalling partners provide a critical barrier to curtail GPA colonization of *Arabidopsis*.

Abbreviations: BIK1, BOTRYTIS-INDUCED KINASE 1; EDS1, ENHANCED DISEASE SUSCEPTIBILTY 1; EIN2, ETHYLENE SENSITIVE 2; EPG , Electrical Penetration Graph; ET , Ethylene; GPA , green peach aphid; LOX5 , LIPOXYGENASE 5; MPL1 , *MYZUS PERSICAE*-INDUCED LIPASE 1; NahG , salicylate hydroxylase gene; PAD4 , PHYTOALEXIN DEFICIENT 4; SA , salicylic acid; SAG , SENESCENCE-ASSOCIATED GENES; SID2 (ICS1) , SALICYLIC-ACID-INDUCTION DEFICIENT 2 (ISOCHORISMATE SYNTHASE 1); SSI2 , SUPPRESSOR OF SALICYLIC ACID INSENSITIVITY 2; TPS11 , TREHALOSE-6-PHOSPHATE SYNTHASE 11; WT , wild type

Keywords: Aphids, *PAD4*, *Arabidopsis*, plant defense.

Introduction

Plants utilize a plethora of defense responses, including molecular and biochemical mechanisms, to protect themselves from various biotic stresses. In *Arabidopsis thaliana*, which has long been used as a model plant to study plant stress response, the *PHYTOALEXIN DEFICIENT 4* (*PAD4*) gene functions as a critical signalling component in defense against various pathogens (Glazebrook, 2005; Wiermer *et al.*, 2005) as well as the green peach aphid (GPA; *Myzus persicae* Sülzer), a phloem sap consuming insect pest that causes considerable damage to a wide variety of plants (Louis *et al.*, 2012*c*; Louis and Shah, 2013). PAD4 interacts with its signalling partner ENHANCED DISEASE SUSCEPTIBILTY 1 (EDS1) to provide resistance against pathogens (Feys *et al.*, 2005). Interaction of PAD4 with EDS1 yields a nucleo-cytoplasmic PAD4–EDS1 complex that promotes accumulation of the plant defense signalling molecule salicylic acid (SA) and regulation of several defense-related genes that contribute to disease resistance. In contrast, the *PAD4*-mediated resistance to the GPA does not require EDS1 or SA (Moran and Thompson, 2001; Mewis *et al.*, 2005; Pegadaraju *et al.*, 2005, 2007; Louis *et al.*, 2012*a*; Lei *et al.*, 2014), thus unveiling a distinct, previously undefined mechanism involving PAD4 in defense against aphid infestation.

PAD4: A key regulator in providing defense against aphid infestation

PAD4 orchestrates antibiotic and antixenotic defenses against aphids. Antibiosis involves mechanisms that influence the physiology of the aphids to adversely affect their growth, development and/or reproduction (Smith, 2005). On the other hand, antixenosis contributes to deterrence of aphid feeding and/or settling on the host plant (Painter, 1951; Kogan and Ortman, 1978). The Electrical Penetration Graph (EPG) technique has provided a useful approach to

study the influence of plant genotypic differences on GPA feeding behavior. EPG analysis confirmed that a PAD4-exerted defense mechanism limits aphid feeding from the sieve elements (Pegadaraju *et al.*, 2007; Louis *et al.*, 2012*a*). The GPA spent significantly longer time in the sieve elements of the *pad4* mutant compared with the wild type (WT) plant. *PAD4* was also required for the accumulation of antibiosis activity. Petiole exudates, which are enriched in vascular sap, collected from the *pad4* mutant were deficient in antibiosis activity compared with the petiole exudates obtained from the WT plants (Louis *et al.*, 2010*a*, 2012*a*).

GPA infestation promotes PAD4 expression in the vascular tissues

GPA infestation resulted in the rapid induction of *PAD4* expression in WT *Arabidopsis* leaves (Pegadaraju *et al.*, 2005 2007; Couldridge *et al.*, 2007; Louis *et al.*, 2010*a*; Lei *et al.*, 2014). Moreover, *PAD4* expression was induced in and around the vascular tissues of GPA-infested leaves (Louis *et al.*, 2012*b*). These results, in conjunction with the EPG studies, suggest that *PAD4* expression in the vasculature is required for limiting GPA colonization. However, *PAD4* expression was also observed in cells other than the vascular tissues (Louis *et al.*, 2012*b*). Thus, a function for PAD4 operating in non-vascular tissues in *Arabidopsis* defense against the GPA cannot be ruled out. Generating transgenic *Arabidopsis* plants that specifically express *PAD4* in the phloem will be useful to further characterize the role of *PAD4* in phloem-based resistance to aphids.

PAD4 promoted senescence contributes to defense against the GPA

Aphids alter host source–sink relationship such that an uninterrupted supply of nutrients is available to the insect. By contrast, senescence acts as a defense mechanism against aphids (Pegadaraju *et al.*, 2005). Leaf senescence results in the removal of nutrients from the aphid-infested leaves, thereby countering the source–sink alterations promoted by aphid colonization. *PAD4* is required for promoting premature leaf senescence in GPA-infested plants, which is characterized by the up-regulation of a subset of *SENESCENCE-ASSOCIATED GENE* (*SAG*) expression, and increased chlorophyll loss and cell death (Pegadaraju *et al.*, 2005; Louis *et al.*, 2012*a*; Lei *et al.*, 2014). The onset of cell death in response to GPA infestation was delayed in the *pad4* mutant, compared with WT plants (Pegadaraju *et al.*, 2005). In contrast, ectopic expression of *PAD4* from the *cauliflower mosaic virus* (*CaMV*) *35S* promoter rapidly induced cell death in response to GPA infestation in the *35S:PAD4* plant (Pegadaraju *et al.*, 2007). Senescence also results in alterations of the redox status (Khanna-Chopra, 2012). A recent study showed that H_2O_2 content increased in GPA-infested *Arabidopsis* leaves, and this increase in H_2O_2 was associated with resistance (Lei *et al.*, 2014). *PAD4* was required for this increase in H_2O_2 in GPA-infested leaves (Lei *et al.*, 2014). These studies suggest that *PAD4*-dependent leaf senescence- and cell death-associated mechanisms potentially contribute to the accumulation of factors that are detrimental for the attacking aphids.

An acyl hydrolase motif is required for PAD4 function in antibiosis and in deterring GPA feeding from the sieve elements

The PAD4 protein contains a triad of Ser (S), Asp (D), and His (H) residues that form the catalytic triad of many α/β fold acyl hydrolases that include lipases (Blow, 1990; Jirage *et al.*, 1999; Feys *et al.*, 2005). However, as yet, no lipase activity has been demonstrated for PAD4. The GPA population size was larger on *Arabidopsis* plants expressing mutant versions of PAD4 [PAD4(S118A) and PAD4(D178A)] in which Ser118 and Asp178 were substituted by Ala, than on WT plants. Furthermore, aphids spent more time in the sieve elements of the PAD4(S118A) compared with WT plants, and petiole exudates collected from the PAD4(S118A) plant lacked the PAD4-regulated antibiosis activity, thus suggesting that S118 is essential for the involvement of PAD4 in providing feeding deterrence and antibiosis activity against aphids (Louis *et al.*, 2012*a*). However, PAD4(S118A) and PAD4(D178A) did not deter insect settling, *SAG* expression, and cell death in response to GPA infestation, thus suggesting the presence of at least two PAD4 containing molecular activities in defense against the GPA (Louis, 2011; Louis *et al.*, 2012*a*).

Host lipids and their relationship with the *PAD4*-mediated defense pathway

Similar to *PAD4*, *MYZUS PERSICAE-INDUCED LIPASE1* (*MPL1*) expression was induced in response to GPA infestation in *Arabidopsis* foliage (Louis *et al.*, 2010*b*). However, unlike *PAD4*, *MPL1* was not required for antixenosis. Like PAD4, the MPL1 protein contains the Ser-Asp-His triad of catalytic site residues that are conserved in α/β fold acyl hydrolases. The MPL1 protein, which exhibits lipase activity, was required only for antibiosis against the GPA (Louis *et al.*, 2010*b*). Whether the lipase activity of MPL1 is indeed required for antibiosis will require additional experiments with plants expressing mutant forms of MPL1 in which the putative catalytic triad amino acid residues have been altered. Comparison of GPA feeding behavior revealed that there was no significant difference in the total amount of time spent by the GPA in the sieve element phase on the *mpl1* null mutant and WT plants, suggesting that the absence of MPL1 function in the *mpl1* mutant does not affect aphid feeding behavior. Petiole exudates of the *mpl1* mutant lacked an antibiosis factor that is present in similar exudates of WT plants. *PAD4* and *MPL1* do not affect the GPA infestation-induced expression of each other (Louis *et al.*, 2010*b*). Furthermore, ectopic expression of *PAD4* and *MPL1* from the *CaMV 35S* promoter in *mpl1* and *pad4* plants, respectively, rescued the antibiosis deficiency of the *mpl1* and *pad4* mutants, indicating that *MPL1* and *PAD4* contribute to two parallel antibiosis mechanisms

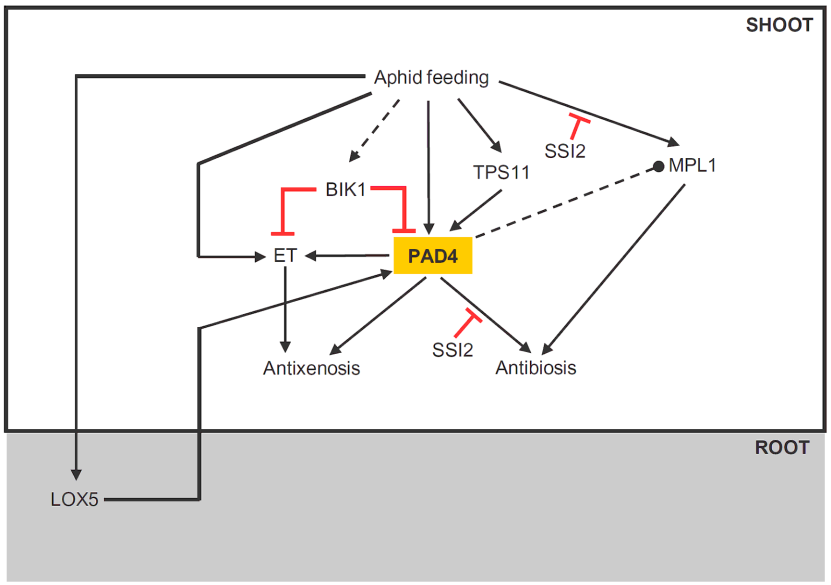

Figure 1. PAD4: A converging point in modulating defense against aphids in *Arabidopsis*. GPA feeding on *Arabidopsis* rapidly activates defense mechanisms, most likely through the host plant perception of aphid salivary elicitors. GPA infestation stimulates expression of *PAD4*, a key defense signalling gene that modulates both antibiotic and antixenotic defenses against the aphid. The GPA infestation-induced up-regulation of *PAD4* expression is regulated by *TPS11* and *LOX5*, which are involved in trehalose and 9-LOX oxylipin metabolism, respectively. *TPS11* expression is up-regulated in *Arabidopsis* shoots upon GPA infestation, whereas GPA feeding on *Arabidopsis* foliage induced accumulation of *LOX5* transcript in the roots. A LOX5 product(s) synthesized in the roots and/or related metabolites are probably translocated to the shoots through the vascular system where they enhance *PAD4* expression. As a parallel defense mechanism, GPA infestation also induces expression of *MPL1*, which encodes a lipase that is associated with antibiosis against the GPA. Both *PAD4* and *MPL1* are required for heightened resistance to GPA in the *ssi2* mutant. Cross-complementation experiments suggest that *MPL1* likely functions independently of *PAD4*. However, available evidence does not rule out the possibility of a *PAD4*-dependent mechanism modulating *MPL1* activity. *BIK1*, a receptor-like cytoplasmic kinase, suppresses *PAD4* expression. Basal expression of *PAD4* is elevated in *bik1* mutants, which exhibit enhanced resistance against the GPA. *PAD4* function is required for the *bik1*-conferred resistance against aphids. Aphid infestation results in ET accumulation, which has been implicated in antixenosis, in particular deterring aphid settling on *Arabidopsis*. The aphid infestation associated emission of ET was elevated in the *bik1* mutant, but not in the *pad4* and the *bik1 pad4* double mutant, thus indicating that *PAD4* is required for the full extent of ET emission and that PAD4's involvement in repelling GPA is probably mediated through ET signalling. [Black lines ending in arrows represent positive effects, broken black lines ending in closed circle represent unknown mechanisms, broken black lines ending in arrow is indicative of constitutive expression, and red lines ending with perpendicular bar indicate repressive effects].

and the elevated levels of one component/mechanism can overcome the deficiency of the other (Figure 1; Louis *et al.*, 2010*b*; J Louis and J Shah, unpublished data). However, the existing evidence does not allow us to rule out the possibility that *PAD4* or a *PAD4*-dependent factor alters the molecular activity of MPL1, and thereby contributes to MPL1-dependent antibiosis against aphids.

Both *PAD4* and *MPL1* contribute to the *suppressor of salicylic acid insensitivity* (*ssi2*)-mediated heightened antibiosis against GPA (Louis *et al.*, 2010*a*, 2010*b*). The *SSI2* gene encodes a plastid-localized stearoyl-ACP desaturase, which catalyses the desaturation of stearic acid to oleic acid and alters the *Arabidopsis* membrane lipid composition (Shah *et al.*, 2001; Kachroo *et al.*, 2001; Nandi *et al.*, 2003). In comparison to the WT plant, the aphid population was significantly reduced in the *ssi2* mutant plant, which exhibits a spontaneous cell death phenotype and accumulates high levels of an antibiosis activity in petiole exudates (Pegadaraju *et al.*, 2005; Louis *et al.*, 2010*a*). *MPL1* expression was constitutively higher in the *ssi2* mutant compared with the WT plant. Furthermore, the heightened antibiosis activity in *ssi2* was dependent on MPL1 function (Pegadaraju *et al.*, 2005; Louis *et al.*, 2010*b*). In contrast to the elevated expression of *MPL1*, basal expression of *PAD4* was not higher in the *ssi2* mutant compared with the WT plants, thus suggesting that *ssi2* likely promotes *PAD4*-dependent antibiosis downstream of *PAD4* transcript accumulation (Figure 1; Louis *et al.*, 2010*a*).

Recently, it was shown that foliar infestation of GPA results in the accumulation of *LIPOXYGENASE 5* (*LOX5*) transcript in roots (Nalam *et al.*, 2013). *LOX5*, which encodes a 9-lipoxygenase, was found to promote aphid colonization. Indeed, the oxylipin 9-hydroxyoctadecadienoic acid (9-HOD) was found to promote aphid colonization on *Arabidopsis* and promote insect fecundity on an artificial diet, thus suggesting that 9-LOX products probably have an effect on the insect (Nalam *et al.*, 2012). Interestingly, *LOX5* was also required for the GPA infestation-associated up-regulation of *PAD4* expression (Nalam *et al.*, 2013). Furthermore, 9-HOD application induced *PAD4* expression in *Arabidopsis* leaves (Nalam *et al.*, 2013), leading to the suggestion that while *Arabidopsis* utilizes LOX5-synthesized products to promote defenses, the GPA has likely evolved to cue on LOX5-derived metabolites to facilitate feeding, growth, and reproduction (Figure 1).

TPS11-dependent trehalose metabolism and *PAD4* interaction in mediating defense against aphids

Trehalose, a non-reducing disaccharide, has a signalling function in plants to protect them from various stresses (Schluepmann *et al.*, 2003; Paul *et al.*, 2008; Fernandez *et al.*,

2010). Trehalose metabolism is also involved in promoting defense against the GPA (Singh *et al.*, 2011; Hodge *et al.*, 2013). In *Arabidopsis*, the *TREHALOSE-6-PHOSPHATE SYNTHASE 11* (*TPS11*) gene is involved in the transient up-regulation of trehalose accumulation in GPA-infested plants. Time-course analysis of *TPS11* transcript accumulation in response to aphid infestation revealed that *TPS11* expression is also transiently up-regulated in GPA-infested leaves and parallels the transient increase in trehalose levels in aphid-infested *Arabidopsis* WT leaves (Singh *et al.*, 2011). Like *PAD4*, *TPS11* also provided antibiotic and antixenotic defenses against the GPA. In addition, EPG analysis revealed that aphids spent more time feeding from the sieve elements of *tps11* null mutant compared with WT plants, thus suggesting that *TPS11* obstructs the aphid's ability to feed uninterruptedly from the sieve elements (Singh *et al.*, 2011).

Trehalose application induced the expression of *PAD4* in *Arabidopsis* WT leaves. Furthermore, the GPA infestation-associated induction of *PAD4* was delayed in the *tps11* null mutant, suggesting a significant contribution of *TPS11* to the timely activation of *PAD4* expression in response to aphid infestation (Singh *et al.*, 2011). In agreement with a function for *TPS11* in promoting *PAD4* expression, higher basal expression of *PAD4* was observed in the *35S*: *TPS11* and *otsB* transgenic plants, which contained elevated levels of trehalose, compared with WT plants. Taken together, the available evidence suggests that *TPS11*-dependent trehalose metabolism contributes to *PAD4*-mediated defense against aphids (Figure 1). However, it was also shown that *TPS11* and trehalose provided defense against aphids, independently of *PAD4*, by modulating carbon metabolism and activating starch accumulation in response to aphid infestation (Singh *et al.*, 2011). It has been suggested that the plants might activate starch accumulation as a counter-defense mechanism to combat aphid attack (Singh *et al.*, 2011).

BIK1, a receptor-like kinase, and its interaction with *PAD4* upon aphid infestation

Very recently, a receptor-like cytoplasmic kinase (RLCK) *BOTRYTIS-INDUCED KINASE 1* (*BIK1*) was shown to control defense against aphids by negatively regulating *PAD4* expression. These receptor-like kinases are elicited when plants are attacked by various microbes and herbivores (Bent and Mackey, 2007; Boller and Felix, 2009; Prince *et al.*, 2014). Unlike *PAD4*, aphid feeding did not significantly induce the expression of *BIK1* in *Arabidopsis* WT leaves (Couldridge *et al.*, 2007; Lei *et al.*, 2014). Relative expression of *BIK1* was comparable between uninfested and aphid-infested WT plants. Loss of *BIK1* function in the *bik1* mutant provided both antibiotic and antixenotic defenses against aphids. In addition, aphids reared on the *bik1* mutants, compared with WT plants, excreted less honeydew, a digestive waste, thus indicating reduced nutrient uptake. The body weight of aphids reared on the *bik1* mutant was also significantly reduced compared with aphids reared on WT plants (Lei *et al*, 2014). Compared with the WT plants, the enhanced resistance against GPA in the *bik1* mutant was accompanied by elevated levels of H_2O_2 accumulation, and enhanced cell death and callose deposition in response to GPA infestation (Lei *et al.*, 2014).

The *bik1*-conferred resistance against the GPA was SA independent. Aphid numbers were comparable between *bik1* and *bik1 sid2* plants or *bik1* and *bik1 nahG* plants, which express the bacterial *NahG*-encoded salicylate hydroxylase that degrades SA and thus does not accumulate elevated levels of SA. Furthermore, comparable aphid numbers were observed on the WT and the SA-deficient *sid2* and *nahG* plants (Lei *et al.*, 2014). It was also shown that GPA infestation induced accumulation of H_2O_2 and cell death in the *bik1* mutant. However, SA was not required for these *bik1*-conferred phenotypes, thus supporting previous studies which inferred that SA was not critical in mediating defense against the GPA (Moran and Thompson, 2001; Mewis *et al.*, 2005; Pegadaraju *et al.*, 2005; Louis *et al.*, 2010*a*).

Basal expression of *PAD4* and *SAG13*, a *PAD4*-regulated senescence-associated gene in *Arabidopsis*, were elevated in the *bik1* mutants compared with the WT plant. Loss of *PAD4* gene function in the *bik1* mutant background compromised the *bik1*-mediated enhanced resistance to GPA. Aphid numbers were significantly higher on *bik1 pad4* double mutant plants than on *bik1* single mutant plants (Lei *et al.*, 2014). Furthermore, aphid feeding induced accumulation of H_2O_2 production and cell death were compromised in *bik1 pad4* plants compared with *bik1* plants (Lei *et al.*, 2014). Taken together, these data suggest that *PAD4* is required for the *bik1*-conferred heightened resistance to aphids.

Studies have shown that ethylene (ET) signalling is required for providing enhanced resistance to aphids (Dong *et al.*, 2004; Anstead *et al.*, 2010; Zhang *et al.*, 2011). Increased aphid repellence on *bik1* mutant plants compared with WT plants at an early time period (6h post release) was mediated through the ET pathway (Lei *et al.*, 2014). Mutations in the *ETHYLENE INSENSITIVE 2* (*EIN2*) gene, a key component in the ET signalling pathway, resulted in attenuation of the *bik1*-conferred deterrence of GPA settling on the *bik1 ein2* double mutant at an early time point compared with the *bik1* single mutant plants (Lei *et al.*, 2014). Similarly, as mentioned before, *bik1 pad4* mutant plants were more attractive to aphids compared with *bik1* plants. Furthermore, aphid infestation resulted in an elevated ET burst in the *bik1* mutant compared with uninfested *bik1* plants. ET release was significantly reduced in *bik1 pad4* plants compared with *bik1* plants before and after aphid infestation, suggesting that *PAD4* is involved in promoting ET accumulation that potentially deters aphid settling on *Arabidopsis*. These results indicate that *BIK1* negatively regulates *PAD4* expression and ET production, whereas the aphid infestation-induced expression of *PAD4* positively modulates the ET emission (Figure 1).

PAD4 beyond *Arabidopsis*

Similar to *Arabidopsis*, in tomato (*Solanum lycopersicum*) plants GPA-infestation up-regulated the expression of *Sl-PAD4*, the tomato homologue of *Arabidopsis PAD4* (Singh and Shah, 2012). Likewise, aphid infestation induced the expression of *SlTPS11* and trehalose application up-regulated *SlPAD4* expression in tomato leaves, thus suggesting that similar defense signalling pathways might be operating in both *Arabidopsis* and tomato. As in *Arabidopsis*, trehalose metabolism likely also contributes to defense against aphids in tomato independent of the *PAD4* pathway by promoting the accumulation of starch that acts a defense mechanism to curtail GPA proliferation (Singh *et al.*, 2011; Singh and Shah, 2012). Additional studies with different host plants are required to confirm how extensively the *PAD4* and trehalose signalling pathways are conserved in providing defense against aphids.

Final remarks

The current evidence indicates that PAD4 is a critical node at which different signals converge to control *Arabidopsis* response to GPA infestation. Negative regulation of *PAD4* expression, presumably by a *BIK1*-dependent mechanism, is likely released and a combination of inductive factors, including trehalose and 9-LOX-derived oxylipins, promote *PAD4* expression in response to GPA infestation, thereby contributing to defenses that limit GPA infestation on *Arabidopsis*. Although the mechanism by which these *PAD4*-activating processes are elicited in response to aphid infestation is unclear, it is likely that the elicitors present in the aphid saliva trigger these mechanisms. Indeed, several recent studies have demonstrated the ability of aphid salivary components to influence plant defense responses (Bos *et al.*, 2010; Atamian *et al.*, 2013; Elzinga *et al.*, 2014; Rodriguez *et al.*, 2014). Although *PAD4* function in defense against the GPA has been studied in *Arabidopsis*, a *PAD4* homologue in tomato was similarly found to respond to GPA infestation as well as trehalose application, thus suggesting that PAD4 function in limiting aphid infestation is likely engaged by plants beyond *Arabidopsis*. However, how PAD4, a nucleo-cytoplasmic protein, modulates host defenses against aphids, and how *PAD4* expression is regulated by *TPS11*-, *LOX5*-, and *BIK1*-dependent mechanisms remains to be unravelled.

Acknowledgments – This work was made possible using start-up funds provided by University of Nebraska-Lincoln to JL and grants from the National Science Foundation (IOS-0919192, MCB-0920600 and MCB-1412942) to JS.

References

Anstead J, Samuel P, Song N, Wu CJ, Thompson GA, Goggin F. 2010. Activation of ethylene-related genes in response to aphid feeding on resistant and susceptible melon and tomato plants. *Entomologia Experimentalis et Applicata* 134, 170–181.

Atamian HS, Chaudhary R, Dal Cin V, Bao E, Girke T, Kaloshian I. 2013. *In planta* expression or delivery of potato aphid *Macrosiphum euphorbiae* effectors Me10 and Me23 enhances aphid fecundity. *Molecular Plant–Microbe Interactions* 26, 67–74.

Bent AF, Mackey D. 2007. Elicitors, effectors, and R genes: The new paradigm and a lifetime supply of questions. *Annual Review of Phytopathology* 45, 399–436.

Blow D. 1990. More of the catalytic triad. *Nature* 343, 694–695.

Boller T, Felix G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* 60, 379–406.

Bos JIB, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA. 2010. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics* 6, e1001216.

Couldridge C, Newbury HJ, Ford-Lloyd B, Bale J, Pritchard J. 2007. Exploring plant responses to aphid feeding using a full *Arabidopsis* microarray reveals a small number of genes with significantly altered expression. *Bulletin of Entomological Research* 97, 523–532.

Dong HP, Peng J, Bao Z, Meng X, Bonasera JM, Chen G, Beer SV, Dong H. 2004. Downstream divergence of the ethylene signalling pathway for harpin-stimulated *Arabidopsis* growth and insect defence. *Plant Physiology* 136, 3628–3638.

Elzinga DA, De Vos M, Jander G. 2014. Suppression of plant defences by a *Myzus persicae* (green peach aphid) salivary effector protein. *Molecular Plant–Microbe Interactions* 27, 747–756.

Fernandez O, Bethencourt L, Quero A, Sangwan RS, Clement C. 2010. Trehalose and plant stress response: friend or foe? *Trends in Plant Science* 15, 409–417.

Feys BJ, Wiermer M, Bhat RA, Moisan LJ, Medina-Escobar N, Neu C, de Cruz-Cabral A, Parker JE. 2005. *Arabidopsis* SENESCENCEASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. *Plant Cell* 17, 2601–2613.

Glazebrook J. 2005. Contrasting mechanisms of defence against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* 43, 205–227.

Hodge S, Ward JL, Beale MH, Bennett M, Mansfield JW, Powell G. 2013. Aphid-induced accumulation of trehalose in *Arabidopsis thaliana* is systemic and dependent upon aphid density. *Planta* 237, 1057–1064.

Jirage D, Tootle TL, Reuber TL, Frost LN, Feys BJ, Parker JE, Ausubel FM, Glazebrook J. 1999. *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signalling. *Proceedings of the National Academy of Sciences, USA* 96, 13583–13588.

Kachroo P, Shanklin J, Shah J, Whittle EJ, Klessig DF. 2001. A fatty acid desaturase modulates the activation of defence signalling pathways in plants. *Proceedings of the National Academy of Sciences, USA* 98, 9448–9453.

Khanna-Chopra R. 2012. Leaf senescence and abiotic stress share reactive oxygen species mediated chloroplast degradation. *Protoplasma* 249, 469–481.

Kogan M, Ortman EF. 1978. Antixenosis: a new term proposed to define Painter's "nonpreference" modality of resistance. *Bulletin of the Entomological Society of America* 24, 175–176.

Lei J, Finlayson S, Salzman RA, Shan L, Zhu-Salzman K. 2014. *BOTRYTIS-INDUCED KINASE1* modulates *Arabidopsis* resistance to green peach aphids via *PHYTOALEXIN DEFICIENT4*. *Plant Physiology* 165, 1657–1670.

Louis J. 2011. Molecular basis of plant defence against aphids: Role of the *Arabidopsis thaliana PAD4* and *MPL1* genes. PhD thesis. University of North Texas.

Louis J, Leung Q, Pegadaraju V, Reese JC, Shah J. 2010a. *PAD4*-dependent antibiosis contributes to the *ssi2*-conferred hyper-resistance to the green peach aphid. *Molecular Plant–Microbe Interactions* 23, 618–627.

Louis J, Lorenc-Kukula K, Singh V, Reese JC, Jander G, Shah J. 2010b. Antibiosis against the green peach aphid requires the *Arabidopsis thaliana MYZUS PERSICAE-INDUCED LIPASE1* gene. *Plant Journal* 64, 800–811.

Louis J, Gobbato E, Mondal HA, Feys BJ, Parker JE, Shah J. 2012a. Discrimination of *Arabidopsis* PAD4 activities in defence against greenpeach aphid and pathogens. *Plant Physiology* 158, 1860–1872.

Louis J, Mondal HA, Shah J. 2012b Green peach aphid infestation induces *Arabidopsis PHYTOALEXIN-DEFICIENT4* expression at site of insect feeding. *Plant Signalling and Behavior* 7, 1–3.

Louis J, Singh V, Shah J. 2012c. *Arabidopsis thaliana*–aphid interaction. *The Arabidopsis Book* 10, e0159.

Louis J, Shah J. 2013. *Arabidopsis thaliana–Myzus persicae* interaction: shaping the understanding of plant defence against phloem-feeding aphids. *Frontiers in Plant Science* 4, 213.

Mewis I, Appel HM, Hom A, Raina R, Schultz JC. 2005. Major signalling pathways modulate *Arabidopsis* glucosinolate accumulation and response to both phloem feeding and chewing insects. *Plant Physiology* 138, 1149–1162.

Moran PJ, Thompson GA. 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defence pathways. *Plant Physiology* 125, 1074–1085.

Nalam VJ, Keeretaweep J, Sarowar S, Shah J. 2012. Root-derived oxylipins promote green peach aphid performance on *Arabidopsis* foliage. *Plant Cell* 24, 1643–1653.

Nalam VJ, Keereetaweep J, Shah J. 2013. The green peach aphid, *Myzus persicae*, acquires a LIPOXYGENASE5-derived oxylipin from *Arabidopsis thaliana*, which promotes colonization of the host plant. *Plant Signalling and Behavior* 8, 1 e22735.

Nandi A, Krothapalli K, Buseman C, Li M, Welti R, Enyedi A, Shah J. 2003. *Arabidopsis sfd* mutants affect plastidic lipid composition and suppress dwarfing, cell death and the enhanced disease resistance phenotypes resulting from the deficiency of a fatty acid desaturase. *Plant Cell* 15, 2383–2398.

Painter RH. 1951. *Insect resistance in crop plants*. New York: Macmillan.

Paul MJ, Primavesi LF, Jhurreea D, Zhang Y. 2008. Trehalose metabolism and signalling. *Annual Review of Plant Biology* 59, 417–441.

Pegadaraju V, Knepper C, Reese JC, Shah J. 2005. Premature leaf senescence modulated by the *Arabidopsis PAD4* gene is associated with defence against the phloem-feeding green peach aphid. *Plant Physiology* 139, 1927–1934.

Pegadaraju V, Louis J, Singh V, Reese JC, Bautor J, Feys B, Cook G, Parker J, Shah J. 2007. Phloem-based resistance to green peach aphid is controlled by *Arabidopsis PHYTOALEXIN DEFICIENT4* without its signalling partner *ENHANCED DISEASE SUSCEPTIBILITY1*. *Plant Journal* 52, 332–341.

Prince DC, Drurey C, Zipfel C, Hogenhout SA. 2014. The leucinerich repeat receptor-like kinase BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 and the cytochrome P450 PHYTOALEXIN DEFICIENT3 contribute to innate immunity to aphids in *Arabidopsis*. *Plant Physiology* 164, 2207–2219.

Rodriguez PA, Stam R, Warbroek T, Bos JIB. 2014. Mp10 and Mp42 from the aphid species *Myzus persicae* trigger plant defences in *Nicotiana benthamiana* through different activities. *Molecular Plant–Microbe Interactions* 27, 30–39.

Schluepmann H, Pellny T, van Dijken A, Smeekens S, Paul M. 2003. Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 100, 6849–6854.

Shah J, Kachroo PK, Nandi A, Klessig DF. 2001. A recessive mutation in the *Arabidopsis SSI2* gene confers SA- and *NPR1*-independent expression of *PR* genes and resistance against bacterial and oomycete pathogens. *Plant Journal* 25, 563–574.

Singh V, Louis J, Ayre B, Reese JC, Shah J. 2011. *TREHALOSE PHOSPHATE SYNTHASE11*-dependent trehalose metabolism regulates *Arabidopsis thaliana* defence against the phloem-feeding insect, *Myzus persicae*. *Plant Journal* 67, 94–104.

Singh V, Shah J. 2012. Tomato responds to green peach aphid infestation with the activation of trehalose metabolism and starch accumulation. *Plant Signalling and Behavior* 7, 1–3.

Smith CM. 2005. *Plant resistance to arthropods: Molecular and conventional approaches*. Dordrecht: Springer.

Wiermer M, Feys BJ, Parker JE. 2005. Plant immunity: The EDS1 regulatory node. *Current Opinion in Plant Biology* 8, 383–389.

Zhang C, Shi H, Chen L, Wang X, Lü B, Zhang S, Liang Y, Liu R, Qian J, Sun W, You Z, Dong H. 2011. Harpin-induced expression and transgenic overexpression of the phloem protein gene *AtPP2-A1* in *Arabidopsis* repress phloem feeding of the green peach aphid *Myzus persicae*. *BMC Plant Biology* 11, 11.