Journal of Insect Physiology 84 (2016) 90–102

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Pseudomonas syringae enhances herbivory by suppressing the reactive oxygen burst in Arabidopsis

Simon C. Groen [a,b,*], Parris T. Humphrey [a], Daniela Chevasco [b], Frederick M. Ausubel [c,d], Naomi E. Pierce [b], Noah K. Whiteman [a,b,*]

[a] *Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, United States*
[b] *Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, United States*
[c] *Department of Genetics, Harvard Medical School, Boston, MA 02115, United States*
[d] *Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, United States*

ARTICLE INFO

Article history:
Received 20 December 2014
Received in revised form 30 June 2015
Accepted 5 July 2015
Available online 21 July 2015

Keywords:
Arabidopsis thaliana
Drosophila melanogaster
Pseudomonas syringae
Scaptomyza
Herbivore
Plant defense
Reactive oxygen species

ABSTRACT

Plant–herbivore interactions have evolved in the presence of plant-colonizing microbes. These microbes can have important third-party effects on herbivore ecology, as exemplified by drosophilid flies that evolved from ancestors feeding on plant-associated microbes. Leaf-mining flies in the genus *Scaptomyza*, which is nested within the paraphyletic genus *Drosophila*, show strong associations with bacteria in the genus *Pseudomonas*, including *Pseudomonas syringae*. Adult females are capable of vectoring these bacteria between plants and larvae show a preference for feeding on *P. syringae*-infected leaves. Here we show that *Scaptomyza flava* larvae can also vector *P. syringae* to and from feeding sites, and that they not only feed more, but also develop faster on plants previously infected with *P. syringae*. Our genetic and physiological data show that *P. syringae* enhances *S. flava* feeding on infected plants at least in part by suppressing anti-herbivore defenses mediated by reactive oxygen species.

1. Introduction

Herbivory has arisen in only a third of living insect orders, but is associated with high rates of diversification when it evolves: nearly 25% of all named species on Earth are herbivorous insects (Farrell, 1998; Mitter et al., 1988). These radiations may be promoted by the dynamics of plant–herbivore interactions, involving the emergence of novel defensive chemicals in plants and counter-adaptations to these defensive compounds in herbivores (Ehrlich and Raven, 1964; Dobler et al., 2012). Although insect-targeting defensive chemicals are often lineage-specific, their induction is regulated in large part by a common defense response pathway mediated by the phytohormone jasmonic acid (JA, see Glossary in Table 1) (Wasternack et al., 2013). Anti-herbivore chemicals are often augmented by generalized plant defense mechanisms, including a protective cuticle, trichomes (Traw and Bergelson, 2003), and the production of reactive oxygen species (ROS) such as oxygen ions and peroxides in a ROS burst induced by herbivore feeding (Collins et al., 2010; Summers and Felton, 1998; Wu et al., 2013). Microbial pathogens and commensals of plants must also overcome these plant defenses (Cao et al., 2012; Melotto et al., 2006; Torres et al., 2006), and the induction and manipulation of the plant immune system by microbial colonizers may have facilitated the evolution of herbivory (McFall-Ngai et al., 2013; Thaler et al., 2012).

The full extent of the influence of microbes on the ecology and evolution of herbivory in insects (excluding vertically transmitted symbionts of hemipterans) is largely unknown (Hansen and Moran, 2014; McFall-Ngai et al., 2013). Bacterial associations are likely to be involved in enhancing herbivore nutrition, but microbes may also be involved in making plants more palatable through detoxification of defensive chemicals (Freeland and Janzen, 1974), or though suppression of their production. In case of the latter, at least two factors are proposed to shape the likelihood and route by which microbes influence herbivory: (1) whether resource acquisition by microbes requires living plant tissue (biotrophy) or the killing of plant tissue (necrotrophy), and (2) whether biotrophic microbes can successfully colonize the host plant (i.e., the microbes are compatible with the host) or not (i.e.,

---

* Corresponding authors at: Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, United States.

E-mail addresses: scgroen@email.arizona.edu (S.C. Groen), pth@email.arizona.edu (P.T. Humphrey), daniela578@gmail.com (D. Chevasco), ausubel@molbio.mgh.harvard.edu (F.M. Ausubel), npierce@oeb.harvard.edu (N.E. Pierce), whiteman@email.arizona.edu (N.K. Whiteman).

http://dx.doi.org/10.1016/j.jinsphys.2015.07.011
0022-1910/

Table 1
Glossary.

Acronyms	
COR	coronatine
DAB	3,3′-diaminobenzidine tetrahydrochloride
ETI	effector-triggered immunity
HR	hypersensitive response
ITC	isothiocyanate
JA	jasmonic acid
JA-Ile	jasmonic acid-isoleucine conjugate
LAM	leaf area mined
OPDA	12-oxophytodienoic acid (JA precursor)
PCD	programmed cell death
PTI	pattern-triggered immunity
ROS	reactive oxygen species
SA	salicylic acid
Arabidopsis thaliana	
aos (or *cyp74a/dde2*)	mutant deficient in accumulation of JA and OPDA
asFBP1.1 (or *H4*)	mutant with knocked-down expression of cell wall peroxidases
coi1-1	mutant insensitive to JA-Ile and COR
CYP81F2	PTI marker gene typically associated with ROS and callose accumulation
ein2-1	mutant insensitive to ethylene
myb51	mutant deficient in accumulation of indole glucosinolates
pad2-1	mutant deficient in accumulation of glutathione
pad4-1	mutant deficient in accumulation of salicylic acid and ethylene
pen4-1 (or *pcs1/cad1*)	mutant deficient in accumulation of phytochelatin
pmr4-1	mutant deficient in accumulation of callose
rbohD	NADPH oxidase mutant
rbohF	NADPH oxidase mutant
sid2-2	mutant deficient in accumulation of salicylic acid
Pseudomonas syringae	
Psm ES4326 *cfa6*	mutant deficient in production of COR
AvrPto, AvrPtoB, HopAI1	*P. syringae* effectors suppressing accumulation of ROS and callose
Scaptomyza flava	
Glutathione S-tranferase D1 (*GstD1*)	xenobiotic metabolism gene important in detoxification
Peritrophin A	integral to peritrophic membrane, physical antioxidant

the interaction is incompatible) (Lazebnik et al., 2014). Compatible biotrophic microbes tend to enhance feeding by chewing herbivores by suppressing the plant immune system with effectors or phytotoxins they deliver into plant cells (Cui et al., 2002; Groen et al., 2013; Lazebnik et al., 2014), whereas necrotrophic fungi and incompatible biotrophic microbes tend to reduce feeding (Cui et al., 2002; Lazebnik et al., 2014). In necrotrophic and incompatible biotrophic interactions, microbial phytotoxins or the recognition of effectors by the plant immune system trigger programmed cell death (PCD) in the plant, which is associated with a hypersensitive response (HR) and the release of ROS and plant defensive chemicals (Cui et al., 2002; Torres et al., 2006; Andersson et al., 2014). Such chemicals have broad-acting toxicity against microbes and animals in addition to their role as signaling molecules.

Herbivory has evolved several times independently in the family Drosophilidae from microbe-feeding ancestors (Lapoint et al., 2013). Herbivorous species in the genus *Scaptomyza*, which is nested within the paraphyletic genus *Drosophila*, generally specialize on plants from the order Brassicales. They overcome the physical barrier of the leaf cuticle by laying eggs in leaf punctures, which adult females create using a dentate ovipositor (Whiteman et al., 2011). The females also feed on sap from these punctures, and this behavior may play a role in oviposition preference. After the eggs hatch, the larvae develop inside the leaves as leafminers, consuming the mesophyll tissue. To some extent adult females and larvae detoxify the lineage-specific defensive chemicals in the Brassicales, the glucosinolate-derived isothiocyanates (ITCs), using the mercapturic acid pathway (Gloss et al., 2014). One key enzyme in this pathway that has been characterized in *Scaptomyza* spp. is Glutathione *S*-transferase D1 (GstD1) (Gloss et al., 2014). Less is known about how *Scaptomyza* spp. larvae respond to the toxic effects of ROS, which can severely damage the peritrophic membrane that lines the midgut and protects insects from microbial attack (Mittapalli et al., 2007c; Summers and Felton, 1998). Evidence from *Drosophila melanogaster*, the Hessian fly, and hemipteran and lepidopteran herbivores suggest that insect herbivores likely employ general xenobiotic metabolism pathways involving Glutathione *S*-transferases (including GstD1) to deactivate ROS species and downstream lipid peroxidation products (Landis et al., 2012; Lei and Zhu-Salzman, 2015; Luan et al., 2013; Mittapalli et al., 2007a,b; Sawicki et al., 2003; Sykiotis and Bohmann, 2008).

The ancestors of the herbivorous drosophilids in the *Scaptomyza* lineage were microbe-feeders, and it is possible that the evolutionary transition to herbivory in this group was influenced in part by bacterial plant colonizers (O'Connor et al., 2014). Indeed, bacterial colonizers of Brassicales inhibit the formation of or detoxify ROS and ITCs, and have evolved several mechanisms to do this (Abramovitch et al., 2003; Fan et al., 2011; Gimenez-Ibanez et al., 2009; Guo et al., 2012; Lewis et al., 2014; Tang et al., 1972; Xiang et al., 2008; Zhang et al., 2007).

A recent survey of *Scaptomyza flava* feeding on wild *Barbarea vulgaris* (Brassicaceae) in Flagstaff, Arizona, found that the fly's gut microbiota closely resemble the leaf microbiota of its host plants (O'Connor et al., 2014). This metamicrobiome is dominated by bacteria from the genus *Pseudomonas*, and encompasses the (hemi)biotrophic phytopathogen *Pseudomonas syringae*. Although *Scaptomyza* and *Pseudomonas* spp. do not strictly depend on one another to colonize plants, some findings suggest that they can engage in a mutually beneficial relationship. *P. syringae* grows epiphytically and apoplastically, and colonizes the leaf after entering through the stomata or wound sites, or through the vasculature after having colonized distal parts of the plant (Hirano and Upper, 2000; Misas-Villamil et al., 2011). Since both *Scaptomyza* spp. adult females and larvae create wound sites during feeding, insect feeding could promote the colonization of leaves by *P. syringae*. Indeed, adult female *S. flava* can act as vectors of *P. syringae* within and between plants (O'Connor et al., 2014). Additionally, adult *S. flava* females feed less and have a lower fecundity after treatment with antibiotics, suggesting that herbivory may be aided by the metabolic potential of gut-inhabiting microbes (O'Connor et al., 2014). These observations were paralleled by results from another field survey of herbivorous *Scaptomyza nigrita* feeding on *Cardamine cordifolia* (Brassicaceae) (Humphrey et al., 2014). The leaf microbiota were similarly dominated by *Pseudomonas* spp., and larvae showed a preference for feeding on plants infected with *P. syringae*. Moreover, in nature, bacterial titers were consistently higher in leaves that had damage from leafmining *S. nigrita*.

Combined with our earlier laboratory studies, where we identified some of the mechanisms by which *P. syringae* can disrupt plant defense against insect herbivores (Cui et al., 2002, 2005; Groen et al., 2013), these observations pose the intriguing possibility that adult and larval *Scaptomyza* spp. may either directly inoculate their host plants with bacteria that facilitate feeding, or indirectly benefit from the defense-suppressive effects of prior *Pseudomonas* spp. infections. This pattern has been observed in other plant–herbivore interactions, in which herbivores benefit from the defense-suppressive effects of the microbes they transmit. These "suppressive" microbes comprise a wide range of obligate pathogens including viruses, phytoplasmas and bacteria (Body et al., 2013; Casteel et al., 2014; Kaiser et al., 2010; Kazan and Lyons,