Plant Signaling & Behavior 8:12, e27592; December; © 2013 Landes Bioscience

Colorado potato beetle manipulates plant defenses in local and systemic leaves

Seung Ho Chung[1], Cristina Rosa[1], Kelli Hoover[1], Dawn S Luthe[2], and Gary W Felton[1,*]

[1]Department of Entomology and Center for Chemical Ecology; The Pennsylvania State University; University Park; PA USA; [2]Department of Plant Science and Center for Chemical Ecology; The Pennsylvania State University; University Park; PA USA

Keywords: Plant-microbe-insect interactions, insect symbionts, induced defenses, systemic response, tomato

Herbivore microbial associates can affect diverse interactions between plants and insect herbivores. Some insect symbionts enable herbivores to expand host plant range or to facilitate host plant use by modifying plant physiology. However, little attention has been paid to the role of herbivore-associated microbes in manipulating plant defenses. We have recently shown that Colorado potato beetle secrete the symbiotic bacteria to suppress plant defenses. The bacteria in oral secretions from the beetle hijack defense signaling pathways of host plants and the suppression of induced plant defenses benefits the beetle's performance. While the defense suppression by the beetle-associated bacteria has been investigated in local damaged leaves, little is known about the effects of the symbiotic bacteria on the manipulation of plant defenses in systemic undamaged leaves. Here, we demonstrate that the symbiotic bacteria suppress plant defenses in both local and systemic tissues when plants are attacked by antibiotic-untreated larvae.

Plants are sessile organisms and cannot escape from insect herbivore attacks. To ward off the threats, plant have evolved diverse range of defense mechanisms to perceive the nature of attackers and mount appropriate defense responses.[1,2] The plant hormone jasmonic acid (JA) and salicylic acid (SA) are main regulators of signaling pathways to efficiently activate defense responses against attackers.[3] Crosstalk between JA and SA defense pathways allows plants to fine-tune defense responses.[4] To counteract induced defense responses, several herbivores secrete effectors to evade or suppress host plant defenses.[5,6] Effectors are delivered to host plants through releasing oral secretions (OS) and few effectors have been identified.[7-10] Manipulation of plant defenses are often associated with negative interaction between JA and SA signaling pathways.[11,12]

Herbivore-associated microbes play important roles in survival and fitness of their hosts by providing nutrients, detoxifying toxins, or protecting against natural enemies.[13,14] A growing body of literature suggests that insect symbionts can facilitate exploitation of host plants and mediate plant-insect interactions.[15] However, little is known how symbiotic microbes manipulate induced defenses against herbivores. Recently, we demonstrated that the larvae of Colorado potato beetle (*Leptinotarsa decemlineata*) deposit the symbiotic bacteria through releasing OS onto wounded leaves to suppress induced defenses in tomato (*Solanum lycopersicum*), which enhances larval performance.[16] We found that untreated larvae with the defense-suppressing bacteria decreased JA-regulated anti-herbivore defense transcripts and enzyme activity but increased an SA-responsive anti-microbial defense transcript. The suppression of induced defenses is mediated by negative crosstalk between JA and SA signaling pathways. We isolated three genera of defense suppressing bacteria in OS and further confirmed that flagellin from one of the bacteria was involved with defense suppression. These data suggest that plants recognize beetle's attacks as microbial so that plants cannot induce effective defense responses against the herbivore.

Plant pathogens activate induced resistance in local and systemic leaves.[17,18] To overcome host plant resistance, some pathogens that are susceptible to SA-regulated defenses induce JA-regulated pathway to suppress SA signaling pathway. It has been shown that coronatine (COR), a mimic of JA, is secreted by several strains of *Pseudomonas syringae* and promotes bacterial infections by suppressing SA-mediated host defenses.[19] Moreover, defense manipulation by the pathogens can occur in local and systemic tissues. For example, infection of virulent *P. syringae* pv *maculicola* ES4326 caused *Arabidopsis* plants susceptible to secondary infection in systemic uninfected leaves, which is mediated by COR.[20] Likewise, it has been well known that insect herbivory triggers induced defenses in damaged (local) and undamaged (systemic) leaves and the defense responses can last several days.[21,22] Although several studies demonstrated herbivores suppress induced defenses in local leaves, there is less attention to the suppression of induced defenses in systemic tissues.

*Correspondence to: Gary W Felton; Email: gwf10@psu.edu

Submitted: 12/02/2013; Accepted: 12/19/2013

Citation: Ho Chung S, Rosa C, Hoover K, Luthe DS, Felton GW. Colorado potato beetle manipulates plant defenses in local and systemic leaves. Plant Signaling & Behavior 2013; 8:e27592; PMID: 24390091; http://dx.doi.org/10.4161/psb.27592

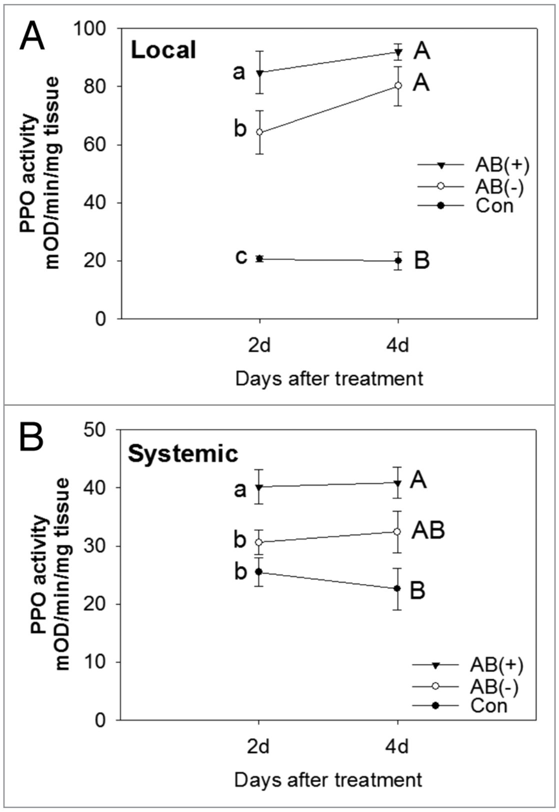

Figure 1. PPO activities in local (**A**) and systemic (**B**) leaves from plants damaged by larvae that fed on AB-treated or untreated leaves. PPO activities were measured 2d and 4d after insect feeding. Values are means ± SEM (n = 5–7). Different letters represent significant differences (ANOVA, $P < 0.05$; followed by LSD test; Local_2d, $F_{(2,15)} = 28.9$, $P < 0.0001$; Local_4d, $F_{(2,16)} = 54.6$, $P < 0.0001$; Systemic_2d, $F_{(2,13)} = 10.2$, $P = 0.0022$; Systemic_4d, $F_{(2,14)} = 7.39$, $P = 0.0066$). Con, undamaged plants; AB(-), plants damaged by untreated larvae; AB(+), plants damaged by AB-treated larvae.

In order to investigate how long the defense suppression mediated by the symbiotic bacteria persists in local and systemic leaves and if the defense suppression can occur in both leaves, we measured PPO activity in plants damaged by untreated or AB-treated larvae. One larva was allowed to feed on the terminal leaflet of 3rd leaves from the bottom for 2–3h. Damaged leaflet (local) and undamaged terminal leaflet (systemic) of 4th leaves were harvested 2d or 4d after the larval feeding. In damaged local leaves, untreated larvae decreased PPO activity compared with AB-treated larvae 2 d after infestation, whereas untreated larvae did not suppress PPO activity 4 d after infestation (**Fig. 1A**). These data indicate that the effect of the symbiotic bacteria on defense suppression is transient. It may be attributed that the symbiotic bacteria deposited onto leaf tissues cannot survive or multiply for 4 d. Thus, the symbiotic bacteria or bacteria-derived effectors may not elicit SA-signaling pathway to antagonize JA signaling pathways for 4 d.

Interestingly, we observed defense suppression by the symbiotic bacteria in undamaged systemic tissues. PPO activity in undamaged leaflets from plants that were damaged by untreated larvae was lower than that in undamaged leaflets from plants that were damaged by AB-treated larvae 2 d after infestation (**Fig. 1B**). However, there was no decrease in PPO activity in undamaged tissues from plants damaged by untreated larvae after 4 d. These data suggest that the suppression of induced defenses by antagonistic interaction between JA and SA signaling pathways can occur in systemic leaves. SA is known to elicit induced resistance in systemic tissues, called systemic acquired resistance (SAR), when infected by biotrophic pathogens.[17] Methyl salicylate (MeSA) is one of the mobile signals from infected tissues to uninfected tissues to induce SAR and MeSA is converted to SA in systemic leaves.[23] Thus, it is possible that SA which is converted from translocated MeSA downregulates JA-signaling pathway in systemic leaves. In addition, JA induced by herbivory functions as a mobile signal to mount induced defenses in systemic leaves.[24] When plants are attacked by untreated larvae, the local tissue may produce lower concentrations of JA than plants attacked by AB-treated larvae. Consequently, the signaling intensity of mobile JA would be low, which cause low levels of PPO activity in systemic leaves from plants damaged by untreated larvae. In summary, we demonstrated the suppression of anti-herbivore defenses by the symbiotic bacteria of CPB in local and systemic tissues. Further study is required to investigate the molecular mechanisms of antagonistic interaction between JA and SA signaling pathways in systemic tissues. It may also be important to determine how long the symbiotic bacteria on leaves persist to manipulate plant defenses. This information helps better understand the complexity of plant-microbe-insect interactions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This research was supported by USDA-NIFA grant 2011–67013–30352 awarded to Felton GW and Luthe DS and NSF IOS-1256326 grant awarded to Felton GW, Rosa C, and Hoover K.

References

1. Howe GA, Jander G. Plant immunity to insect herbivores. Annu Rev Plant Biol 2008; 59:41-66; PMID:18031220; http://dx.doi.org/10.1146/annurev.arplant.59.032607.092825
2. Wu J, Baldwin IT. New insights into plant responses to the attack from insect herbivores. Annu Rev Genet 2010; 44:1-24; PMID:20649414; http://dx.doi.org/10.1146/annurev-genet-102209-163500
3. Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM. Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 2012; 28:489-521; PMID:22559264; http://dx.doi.org/10.1146/annurev-cellbio-092910-154055
4. Thaler JS, Humphrey PT, Whiteman NK. Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 2012; 17:260-70; PMID:22498450; http://dx.doi.org/10.1016/j.tplants.2012.02.010
5. Hogenhout SA, Bos JIB. Effector proteins that modulate plant—insect interactions. Curr Opin Plant Biol 2011; 14:422-8; PMID:21684190; http://dx.doi.org/10.1016/j.pbi.2011.05.003
6. Walling LL. Adaptive defense responses to pathogens and insects. In: Van Loon LC, ed. Adv. Bot. Res. London: Academic Press; 2009, 551-612.
7. Chung SH, Felton GW. Specificity of induced resistance in tomato against specialist lepidopteran and coleopteran species. J Chem Ecol 2011; 37:378-86; PMID:21455676; http://dx.doi.org/10.1007/s10886-011-9937-0
8. Bos JIB, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA. A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). PLoS Genet 2010; 6:e1001216; PMID:21124944; http://dx.doi.org/10.1371/journal.pgen.1001216

9. Felton GW, Tumlinson JH. Plant-insect dialogs: complex interactions at the plant-insect interface. Curr Opin Plant Biol 2008; 11:457-63; PMID:18657469; http://dx.doi.org/10.1016/j.pbi.2008.07.001
10. Wu S, Peiffer M, Luthe DS, Felton GW. ATP hydrolyzing salivary enzymes of caterpillars suppress plant defenses. PLoS One 2012; 7:e41947; PMID:22848670; http://dx.doi.org/10.1371/journal.pone.0041947
11. Musser RO, Hum-Musser SM, Eichenseer H, Peiffer M, Ervin G, Murphy JB, Felton GW. Herbivory: caterpillar saliva beats plant defences. Nature 2002; 416:599-600; PMID:11948341; http://dx.doi.org/10.1038/416599a
12. Weech M-H, Chapleau M, Pan L, Ide C, Bede JC. Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. J Exp Bot 2008; 59:2437-48; PMID:18487634; http://dx.doi.org/10.1093/jxb/ern108
13. Douglas AE. Microbial brokers of insect-plant interactions revisited. J Chem Ecol 2013; 39:952-61; PMID:23793897; http://dx.doi.org/10.1007/s10886-013-0308-x
14. Hansen AK, Moran NA. The impact of microbial symbionts on host plant utilization by herbivorous insects. Mol Ecol 2013; PMID:23952067; http://dx.doi.org/10.1111/mec.12421
15. Feldhaar H. Bacterial symbionts as mediators of ecologically important traits of insect hosts. Ecol Entomol 2011; 36:533-43; http://dx.doi.org/10.1111/j.1365-2311.2011.01318.x
16. Chung SH, Rosa C, Scully ED, Peiffer M, Tooker JF, Hoover K, Luthe DS, Felton GW. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc Natl Acad Sci U S A 2013; 110:15728-33; PMID:24019469; http://dx.doi.org/10.1073/pnas.1308867110
17. Durrant WE, Dong X. Systemic acquired resistance. Annu Rev Phytopathol 2004; 42:185-209; PMID:15283665; http://dx.doi.org/10.1146/annurev.phyto.42.040803.140421
18. Mishina TE, Zeier J. The Arabidopsis flavin-dependent monooxygenase FMO1 is an essential component of biologically induced systemic acquired resistance. Plant Physiol 2006; 141:1666-75; PMID:16778014; http://dx.doi.org/10.1104/pp.106.081257
19. Zhao Y, Thilmony R, Bender CL, Schaller A, He SY, Howe GA. Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. Plant J 2003; 36:485-99; PMID:14617079; http://dx.doi.org/10.1046/j.1365-313X.2003.01895.x
20. Cui J, Bahrami AK, Pringle EG, Hernandez-Guzman G, Bender CL, Pierce NE, Ausubel FM. *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores. Proc Natl Acad Sci U S A 2005; 102:1791-6; PMID:15657122; http://dx.doi.org/10.1073/pnas.0409450102
21. Green TR, Ryan CA. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 1972; 175:776-7; PMID:17836138; http://dx.doi.org/10.1126/science.175.4023.776
22. Rodriguez-Saona CR, Musser RO, Vogel H, Hum-Musser SM, Thaler JS. Molecular, biochemical, and organismal analyses of tomato plants simultaneously attacked by herbivores from two feeding guilds. J Chem Ecol 2010; 36:1043-57; PMID:20820890; http://dx.doi.org/10.1007/s10886-010-9854-7
23. Park S-W, Kaimoyo E, Kumar D, Mosher S, Klessig DF. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 2007; 318:113-6; PMID:17916738; http://dx.doi.org/10.1126/science.1147113
24. Schilmiller AL, Howe GA. Systemic signaling in the wound response. Curr Opin Plant Biol 2005; 8:369-77; PMID:15939667; http://dx.doi.org/10.1016/j.pbi.2005.05.008