

Changes of feeding behavior and salivary proteome of Brown Marmorated Stink Bug when exposed to insect-induced plant defenses

Serteyn Laurent¹, Ponnet Lola¹, Saive Matthew², Backus Elaine A.³, Fauconnier Marie-Laure², Francis Frédéric¹

¹ Laboratory of Functional and Evolutionary Entomology - Gembloux Agro-Bio Tech - University of Liege (Belgium)

² General and Organic Chemistry - Gembloux Agro-Bio Tech - University of Liege (Belgium)

³ USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center (USA)

E-mail : laurent.serteyn@uliege.be

Introduction

Halyomorpha halys Stål (Heteroptera, Pentatomidae), the Brown Marmorated Stink Bug (BMSB), is native to Eastern Asia, where it feeds on a large diversity of host plants. BMSB has been accidentally introduced in Switzerland, Europe, where first observations occurred in 2007. It is probable that the pest will have colonized a large part of Europe within the next decades. Therefore it is crucial to better understand its biology to develop efficient control strategies.

Phytophagous Pentatomidae use different feeding strategies according to the plant tissue. On seeds, they apply a cell rupturing strategy, while on leaves and stems, they secrete a salivary sheath to facilitate the penetration of the stylets through the cells. Regarding their feeding strategies, they are more likely to induce mainly the jasmonic acid pathway as a plant defensive response. Yet, there is a lack of knowledge concerning the behavioral and physiological response of an insect exposed to such an elicited plant.

Objectives

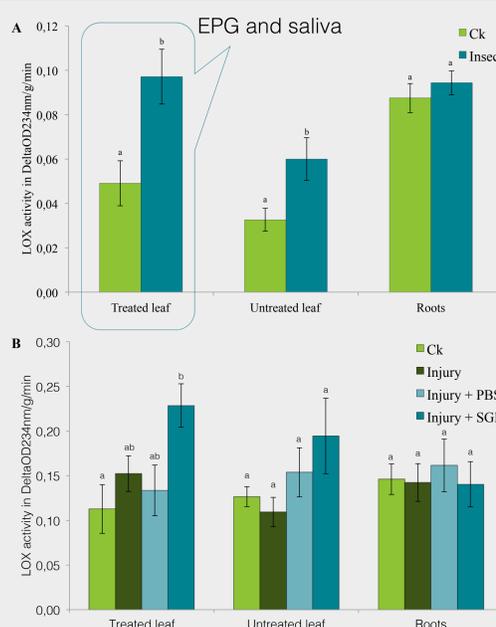
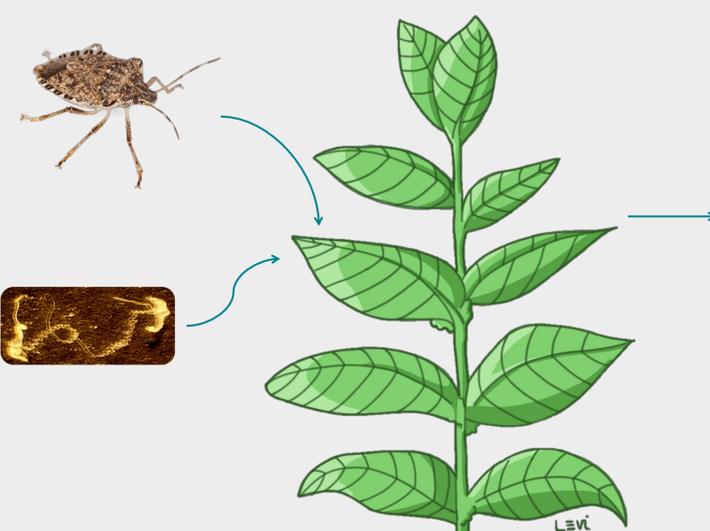
We hypothesize that BMSB invasiveness and wide host range are permitted by the ability of the pest to overcome the defense that it itself induces.

We therefore aimed to enlighten the interactions between BMSB and one of its host plants, *Vicia faba* L., focusing on:

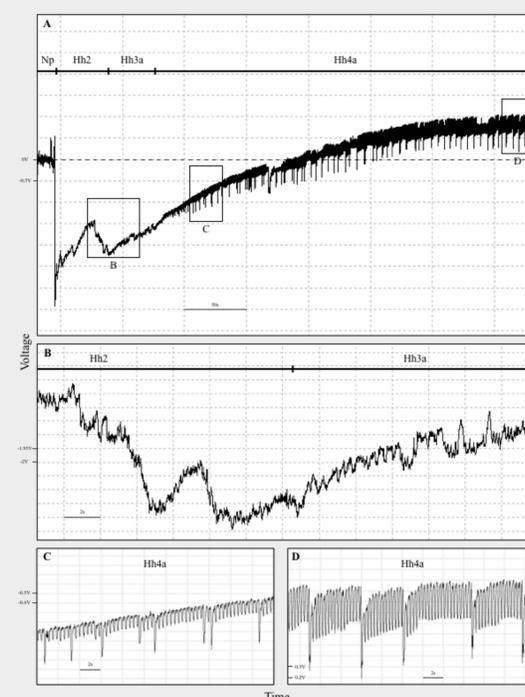
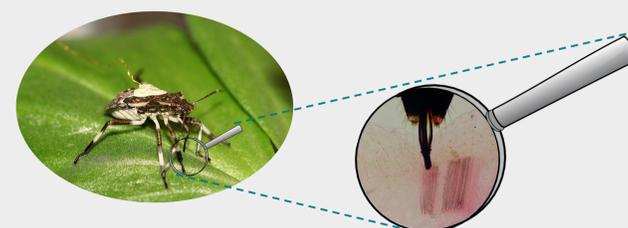
(1) the validation of the hypothesis that JA pathway is induced by this insect feeding and/or its salivary compounds, both locally and systemically;

(2) whether other individuals are subsequently able to detect that response and adapt their feeding strategy.

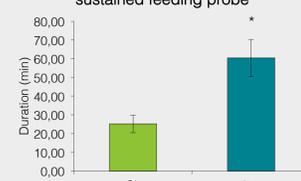
Plant defensive response: lipoxygenase (LOX) activity



Insect feeding behavior: electropenetrography (EPG)

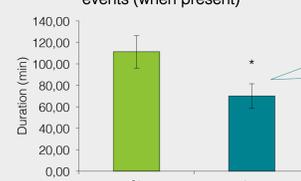


Time from the start to the 1st sustained feeding probe



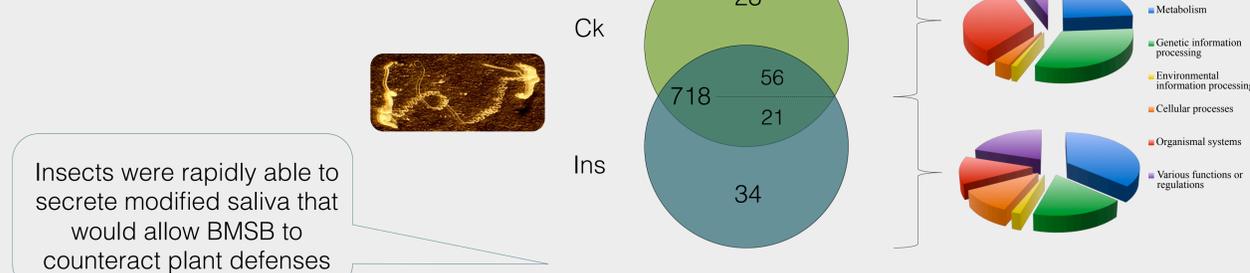
Insects placed on an elicited leaf took a longer time to start probing.

Duration of sustained ingestion events (when present)



And when they finally feed sustainably, it globally lasted less time than for insects on naive plants.

Salivary compounds: gel-free quantitative proteomics



Insects were rapidly able to secrete modified saliva that would allow BMSB to counteract plant defenses

Fasta headers	Secretion signal peptide	Log2(mean LFQ intensity)			Biological process	Putative role in plant-insect interactions
		Ck	Ins	P value		
Down-regulated in "Ins" treatment						
>XP_014291184.1 AQM58360.1 venom protein family 2 protein 3 [Prithesancus plagiipennis]	Yes	28.89	NS		Secretome	Toxin activity
>XP_014286982.1 endophilin-B1 isoform X4 [Halyomorpha halys]	No	24.98	NS		Various regulations	Cellular response to starvation
Quantitative differences						
>XP_014283902.1 glutathione S-transferase isoform X2 [Halyomorpha halys]	No	30.02	29.38	0.010	Stress response	Response to oxidative stress
>XP_014278512.1 ATU82686.1 venom protein family 10 protein 2 [Prithesancus plagiipennis]	Yes	30.75	30.26	0.011	Secretome	Toxin activity
>XP_014281281.1 ATU82691.1 venom protein family 12 protein 1b [Prithesancus plagiipennis]	Yes	28.63	27.46	0.007	Secretome	Toxin activity
>XP_014280129.1 vasostab-like [Halyomorpha halys]	Yes	27.77	26.41	0.020	Secretome	Toxin activity
>XP_014292139.1 probable GPI-anchored adhesion-like protein PGA18 isoform X2 [Halyomorpha halys]	Yes	30.74	29.75	0.038	Structural molecule	Pathogenesis
Up-regulated in "Ins" treatment						
>XP_014276614.1 carboxyl reductase [NADPH] 3-like [Halyomorpha halys]	No	NS	26.15		Carbohydrate metabolism	Xenobiotic metabolic process
>XP_014280077.1 glycogen (starch) synthase [Halyomorpha halys]	No	NS	25.26		Carbohydrate metabolism	Cellular response to starvation
>XP_024218988.1 guanine deaminase isoform X2 [Halyomorpha halys]	No	NS	27.05		Carbohydrate metabolism	Production of xanthine
>XP_014278522.1 carboxypeptidase Q-like [Halyomorpha halys]	Yes	NS	25.96		Protein metabolism	Sensory perception of smell
>BAN20936.1 peroxiredoxin [Riptortus pedestris]	Yes	NS	28.21		Stress response	Response to oxidative stress
>XP_024214585.1 alpha.alpha.-trehalose-phosphate synthase [UDP-forming] [Halyomorpha halys]	No	NS	24.41		Stress response	Cellular response to oxidative stress
>XP_024217780.1 KOC60175.1 UDP-glucuronosyltransferase 1-6 [Habropoda laboriosa]	No	NS	27.74		Stress response	Xenobiotic metabolic process
>XP_014288771.1 neural/ectodermal development factor IMP-12 [Halyomorpha halys]	Yes	NS	25.27		Biological rhythm	Response to starvation
>XP_024214976.1 cubitin-like [Halyomorpha halys]	Yes	NS	24.61		Various functions	Hyperosmotic response
>XP_014282104.1 flotillin-1 [Halyomorpha halys]	No	NS	23.73		Various regulations	Cellular response to exogenous dsRNA
>XP_014283980.1 protein AXTH-like [Halyomorpha halys]	No	NS	22.37		Various regulations	Negative regulation of reactive oxygen species metabolic process and of superoxide anion generation
>XP_024219894.1 esterase PE4-like, partial [Halyomorpha halys]	No	27.84	28.64	0.034	Amino acid metabolism	Resistance to organophosphate insecticides
>XP_014281850.1 ATP-binding cassette sub-family F member 1 [Halyomorpha halys]	No	26.33	27.12	0.006	Protein metabolism	Inflammatory response
>XP_014283513.1 xanthine dehydrogenase/oxidase-like isoform X1 [Halyomorpha halys]	No	26.66	28.20	0.016	Various regulations	Positive regulation of reactive oxygen species metabolic process

Conclusions

This is the first time that a comparative study associates insect's feeding behavior and its salivary compounds investigation, even if both aspects cannot be separated from each other. With this approach, we were able to lessen the hypotheses resulting from each aspect separately, and draw stronger conclusions. Our results suggest that BMSB is able to recognize plant defenses and rapidly adapt its salivary compounds, which would allow a remarkable plasticity of host plants. However, despite the gregarious behavior of BMSB, individuals seemed to be negatively impacted by plant defense induced by their own presence. They struggled to counteract allelochemicals, adapting their feeding behavior and their salivary compounds. Therefore, while foraging for food in nature, they would tend to avoid previously infested plants, which would lead to greater damage and propagation of the invasive pest. In any case, this study identified behavioral and physiological traits of this new pest species, providing novel insights on how it interacts with host plants.