Insight into feeding behaviour and salivary proteome of two pentatomids

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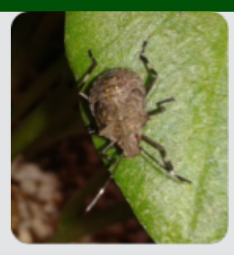
The Brown Marmorated Stink Bug (BMSB)



Halyomorpha halys Stål, the Brown Marmorated Stink Bug (BMSB), is native to Eastern Asia. It has been accidentally introduced in Switzerland, where first observations occurred in 2007. It is probable that the pest will have colonized a large part of Europe within the next decades.

BMSB is phytophagous, feeding on various structures but preferring fruits. It is highly polyphagous in its native region and the most of its host plants are also present in Europe. Therefore, BMSB could easily find woody hosts, crop fields, or perennial herbaceous plants in their areas of introduction. That polyphagy is surely contributing to its colonization process throughout the world, because it is a characteristic of many invasive species

Electrophysiology of feeding behavior

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 stylet (Backus 1988).

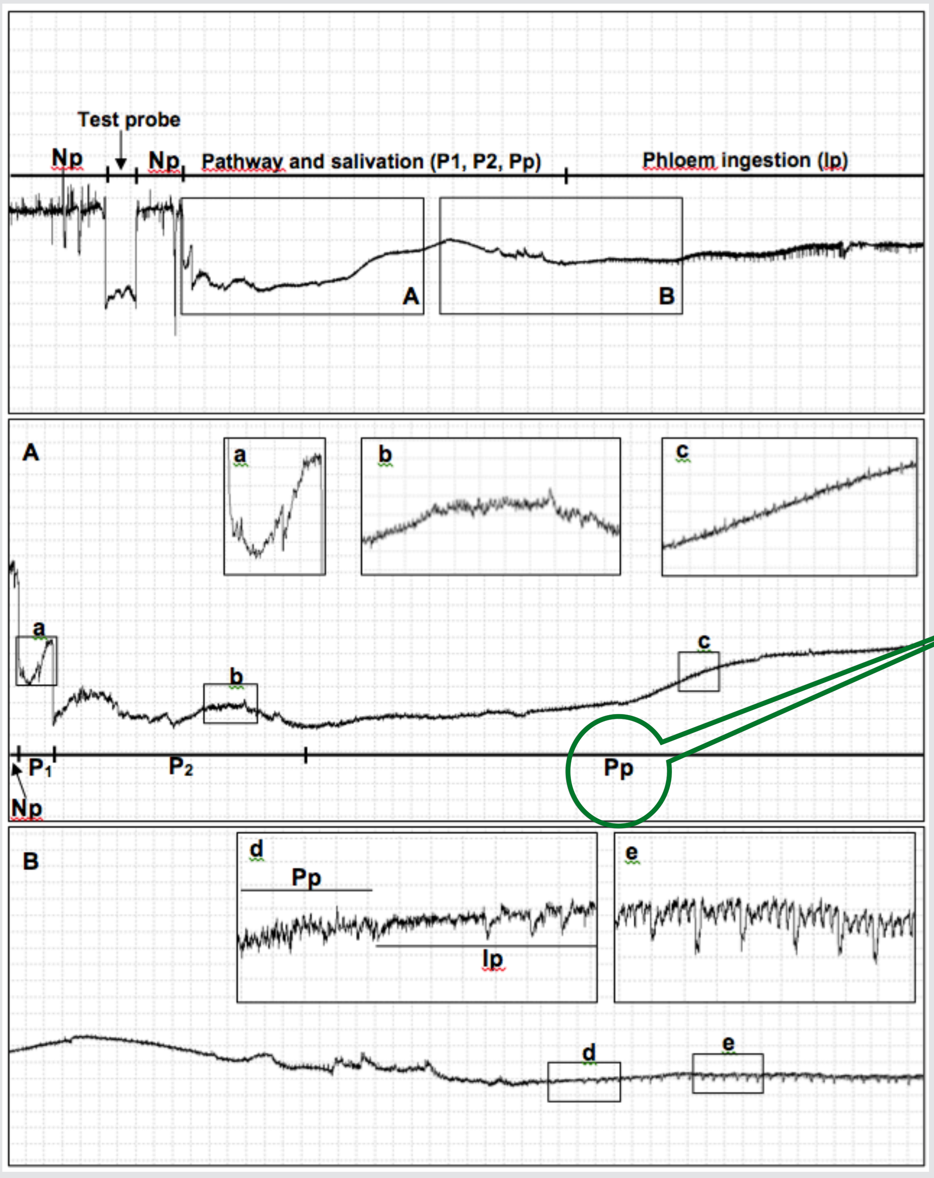
Electrical Penetration Graph (EPG) is a well-known tool, widely used in studies on aphids or whiteflies. EPG allows analysis of precise feeding behaviours, such as pathway, salivation, phloem or xylem ingestion phases… Only a few EPG studies have been published with Heteroptera (Backus et al. 2013; Lucini and Panizzi 2016), and none on BMSB.

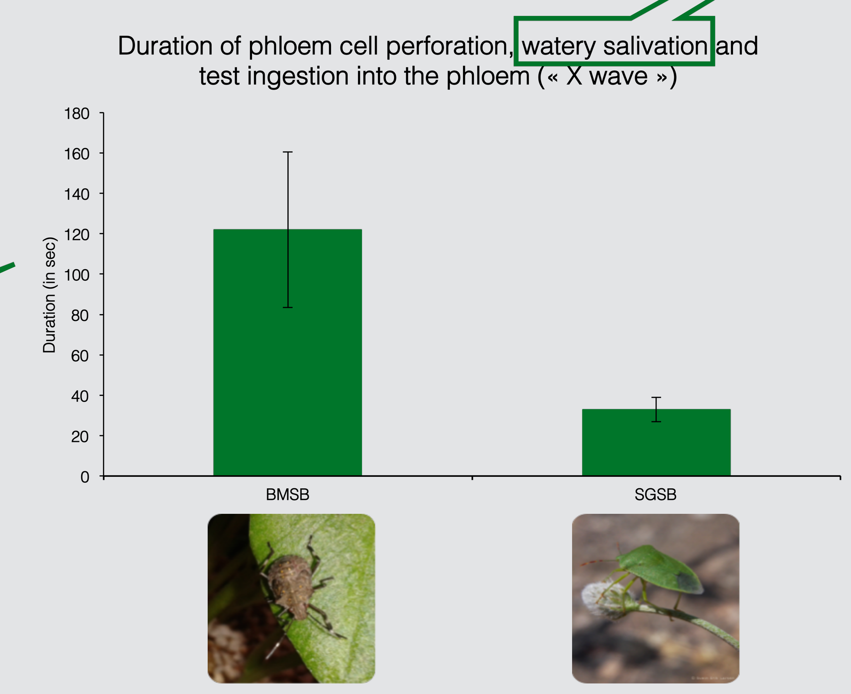
Objectives

In order to investigate the behavioural and physiological traits involved in polyphagy, we led a comparative study between BMSB and the less generalist Southern Green Stink Bug (SGSB), *Nezara viridula* L., on faba bean plants (*Vicia faba* L.), focusing on their feeding behaviour and salivary proteome.

Feeding behaviour of BMSB and SGSB

The following figure presents typical EPG waveforms and their interpretation based on Lucini and Panizzi (2016) and Backus et al. (2013). Then we propose a list of parameters that can be used to set a comparative study based on traits potentially involved in damage and pest adaptation to the plant.





Behaviour			Waveform	Code	Parameters	BMSB (n=8)	SGSB (n=10)	P-value
Non-probing			No waveform at 0V or irregular waveforms with high amplitudes	Np	Total duration (in s) of non-probing behavior before the probe of the first phloem ingestion	741,23 ± 526,95	598,81 ± 218,00	0,412 NS
Test probes			Voltage drop then variable frequencies and amplitudes for more than 1sec	P1 and P2	Time (in s) elapsed before the first test probe	229,75 ± 192,65	47,46 ± 15,93	0,367 NS
					Number of test probes before the probe of the first phloem ingestion	6,83 ± 1,62	10,00 ± 2,61	0,381 NS
					Mean duration (in s) of test probes before the probe of the first phloem ingestion	13,58 ± 6,27	21,12 ± 5,60	0,489 NS
First phloem-ingestion probe	Perforation of leaf epidermis		Voltage drop	/	Time elapsed (in s) before the probe of the first phloem ingestion	821,81 ± 382,53	927,02 ± 282,68	0,410 NS
	Pathway through leaf tissue	Salivary sheath secretion	Variable frequencies and low amplitudes	P1 and P2	Duration (in s) of the salivary sheath secretion and pathway phase	50,65 ± 16,07	36,75 ± 10,67	0,352 NS
		Phloem cells perforation, watery salivation and test ingestion	Constant rise of voltage, with very low amplitude ("X-wave")	Pp	Duration (in s) of phloem cell penetration, watery salivation and test ingestion of the phloem (« X wave »)	121,93 ± 38,57	32,89 ± 6,05	0,015 *
	Ingestion		Regular alternation of plateaus and valleys	Ip	Duration (in s) of the first phloem ingestion	3306,45 ± 638,60	3921,04 ± 826,97	0,580 NS

Salivary proteome

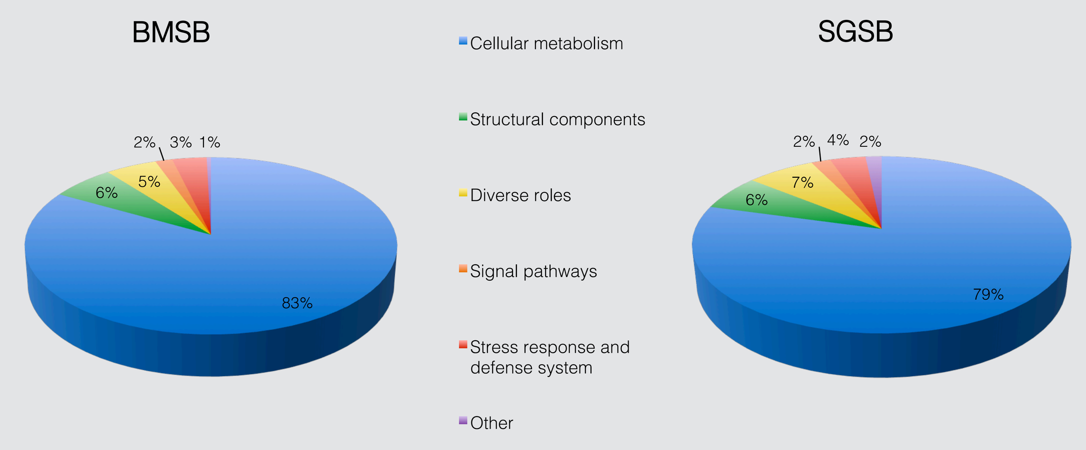
Salivary glands dissection, protein extraction and liquid chromatography coupled with mass spectometry (LC-ESI-MS/MS).

→ 235 and 305 proteins were identified for BMSB and SGSB, respectively.

Differential proteins with a known direct role in response to host plant defense compounds

Protein	Organism	Accession number	MW	pI	Mowse score	*N. viridula*	*H. halys*	Putative function in interactions
serine/threonine-protein phosphatase PP-V	*Pediculus humanus*	gi|212510897	35465	5,3	137	X		Response to oxidative stress, among other functions
protein SZT2	*Bombus terrestris*	gi|340728997	376822	6,02	41	X		Putative response to oxidative stress
xanthine dehydrogenase	*Anopheles gambiae*	gi|157016103	135474	8,36	39		X	Detoxification of plant defense compounds
thioredoxin reductase 1, mitochondrial	*Drosophila persimilis*	gi|194117352	55791	7,57	36		X	Response to oxidative stress

Comparison based on proteins biological function



- We highlighted a similar feeding behaviour on faba bean leaves between the two pentatomids. The only observed difference is the longer "X wave" for BMSB, which could result in a higher amount of effector proteins injected into the phloem and therefore a better adaptation to various host plants. However, these results would benefit from a description of complete waveforms libraries. We should also add an oligophagous stink bug and more replicates, in order to get a more reliable interpretation.
- Even if we identified xanthine dehydrogenase as a candidate effector protein for BMSB, we would recommend a quantitative proteomic analysis, because the salivary glands proteomes are too close between these two insects in a qualitative point of view. Also, it would be better to work on saliva actually injected into phloem sieve elements, following the protocol of Peiffer and Felton (2014).

Backus EA (1988) Sensory systems and behaviours which mediate hemipteran plant-feeding: A taxonomic overview. J Insect Physiol 34(3):151–165. doi: 10.1016/0022-1910(88)90045-5
Backus EA, Rangasamy M, Stamm M, et al (2013) Waveform library for chinch bugs (Hemiptera : Heteroptera : Blissidae) : Characterization of electrical penetration graph waveforms at multiple input impedances. Ann Entomol Soc Am 106(4):524–539.
Lucini T, Panizzi AR (2016) Waveform characterization of the soybean stem feeder Edessa meditabunda : overcoming the challenge of wiring pentatomids for EPG. Entomol Exp Appl 158(2):118–132. doi: 10.1111/eea.12389
Peiffer M, Felton G (2014) Insights into the saliva of the brown marmorated stink bug Halyomorpha halys (Hemiptera: Pentatomidae). PLoS One 9(2):e88483. doi: 10.1371/journal.pone.0088483

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