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Louise Huot, Simon George, Pierre-Alain Girard, Marc Ravallec, Nicolas Negre, et al.. Spodoptera frugiperda tissue-specific transcriptional response to infestation by Steinernema carpocapsae. Congrès IMMUNINV 2017, Jun 2017, Lyon, France. hal-02154485

HAL Id: hal-02154485

<https://hal.science/hal-02154485>

Submitted on 5 Jun 2020

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Spodoptera frugiperda tissue-specific transcriptional response to infestation by Steinernema carpocapsae

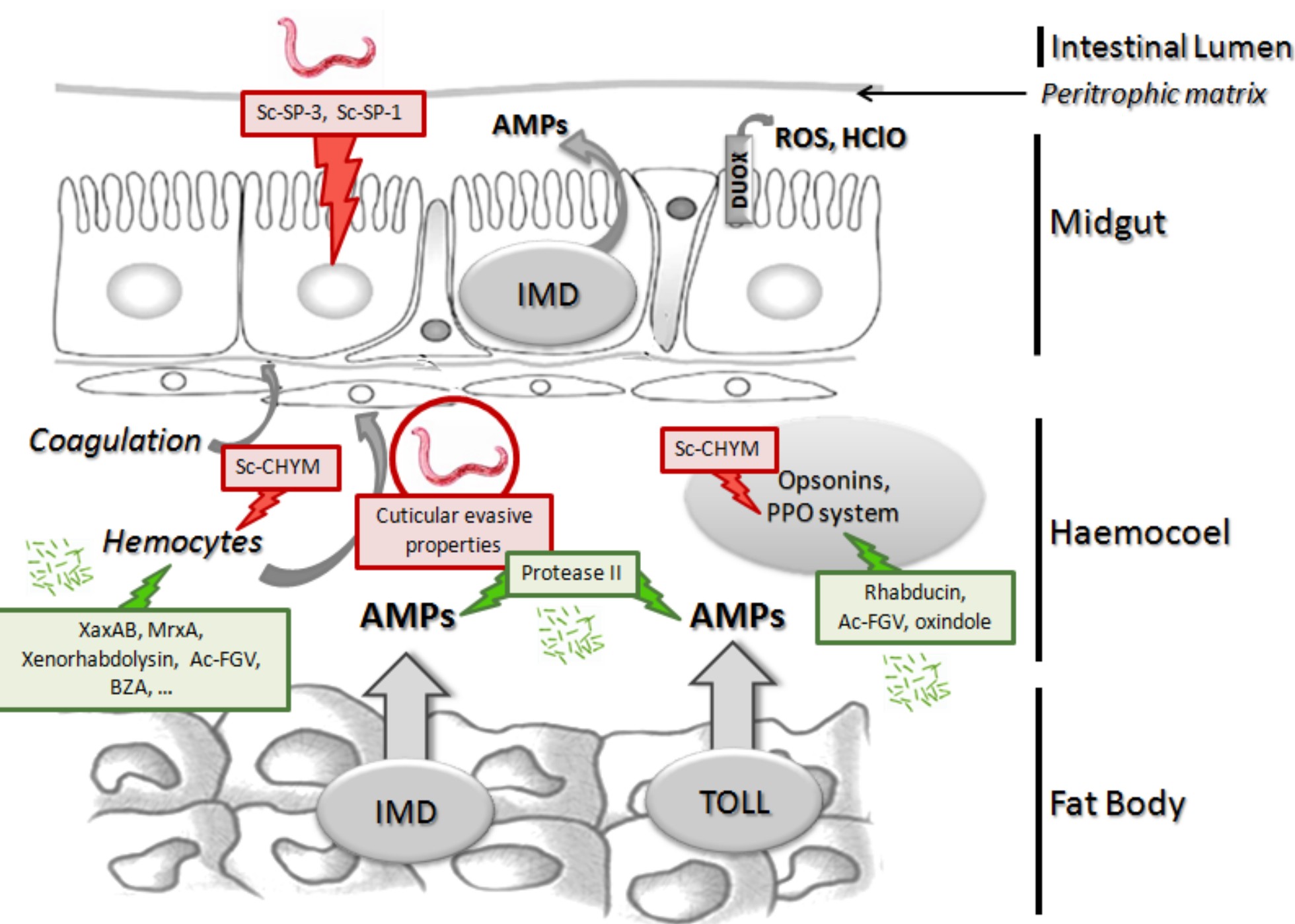
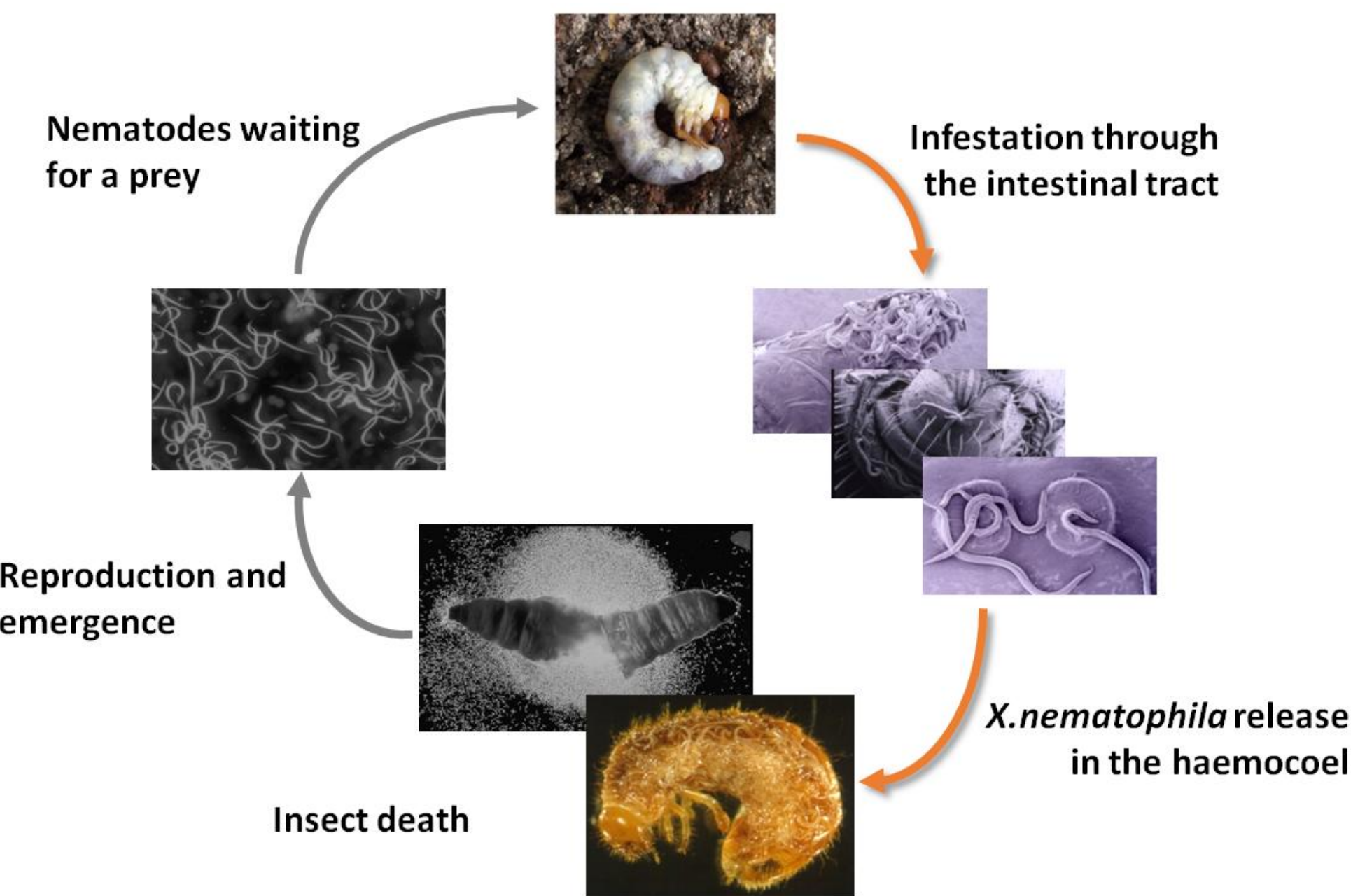


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Context

Steinernema carpocapsae is an entomopathogenic nematode used as biocontrol agent against diverse agricultural pests. When *S. carpocapsae* meets a prey, it enters the insect's haemocoel through the intestinal tract. Once in the haemocoel, the nematode releases its intestinal bacterial symbiont *Xenorhabdus nematophila*. Then, the partners act together to kill their host in 24 to 48 hours by septicemia and toxemia. To finish, *S. carpocapsae* and *X. nematophila* feed on the insect's tissues to multiply for weeks, after what they reassociate and leave the insect's cadaver.



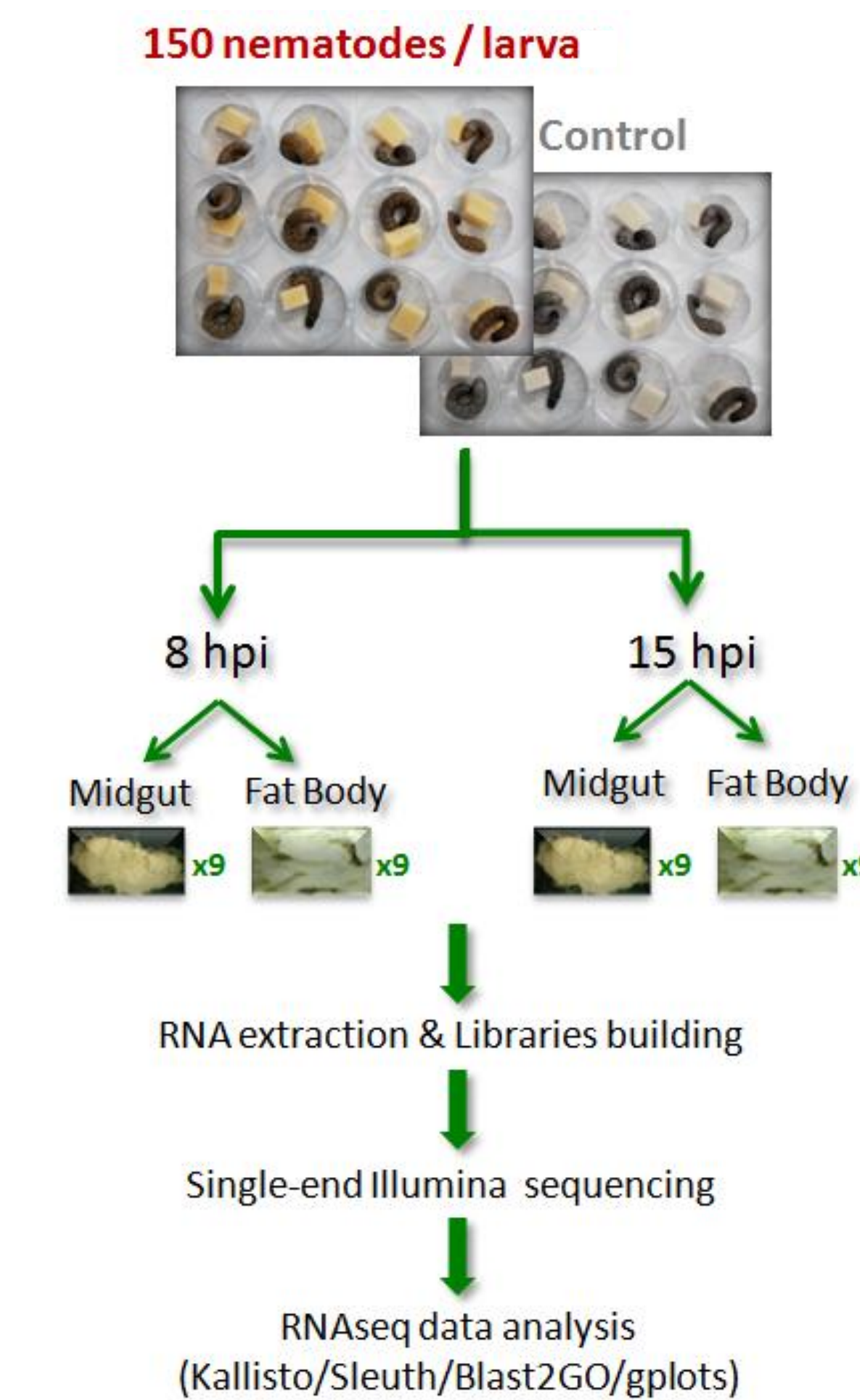
Getting over the insect's immune system is an important step in the establishment of parasitism. Over the past decades, many nematobacterial complex virulence effectors with immunosuppressive and immunoevasive properties have been identified. Nevertheless, only few studies have aimed to describe the immune response that is produced by insect hosts after infestation by entomopathogenic nematodes. Most of them consisted in whole *Drosophila melanogaster* larvae RNAseq experiments. They show that the bacterial symbiont induces a strong humoral immune response whereas the aposymbiotic nematode seems to mostly induce the expression of developmental and coagulation-related genes.

Main Goals

In this study, we explored for the first time tissue-specific transcriptional responses to the infestation by an entomopathogenic nematode in a lepidopteran pest model. Our main goals were to :

- Make the connection between the infectious process and the insect's physiological and immune responses
- Accurately describe the immune response to infestation of a lepidopteran insect

Methods



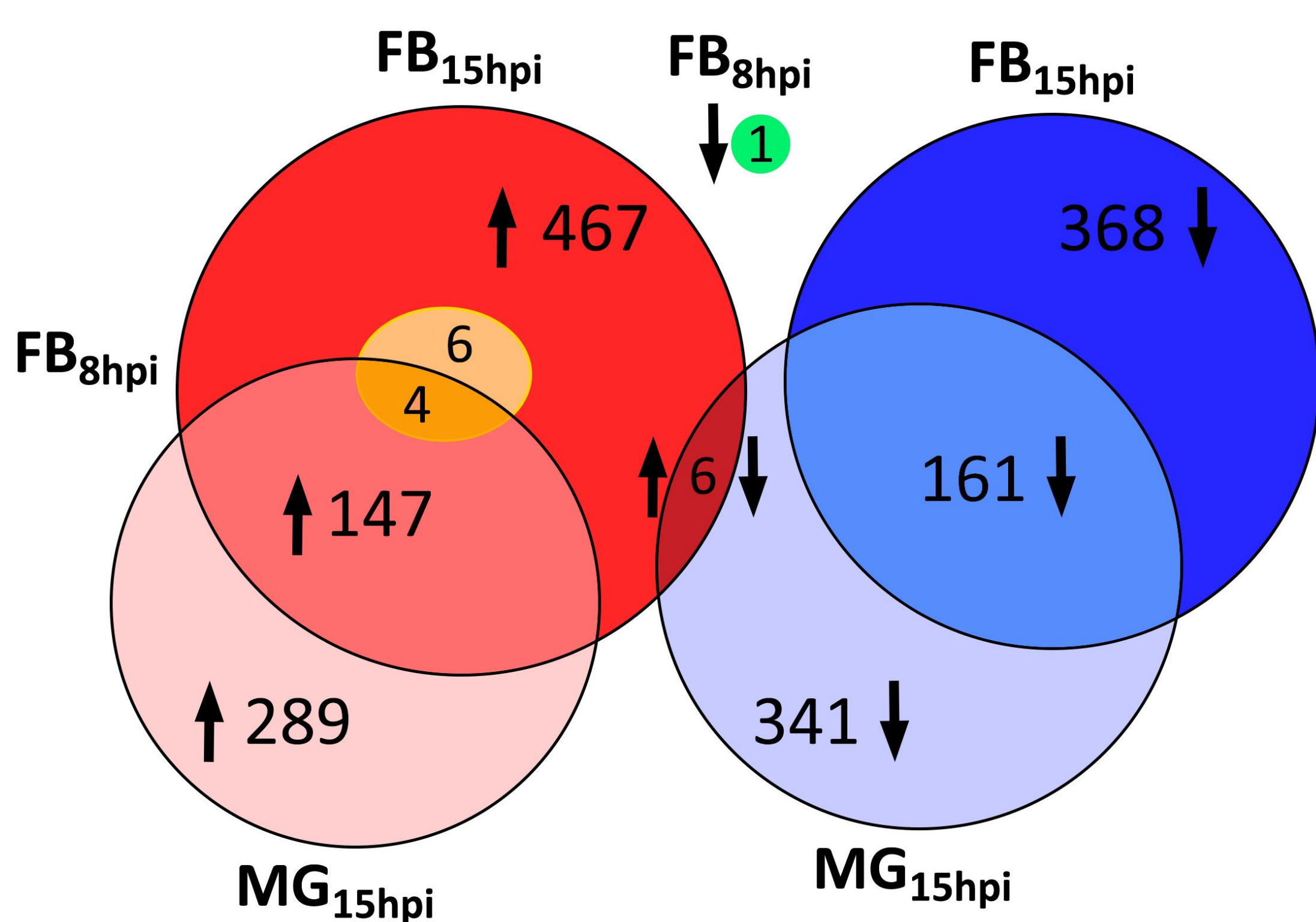
We explored the transcriptional responses at two times :
• 8 hpi, a time close to the nematode entrance in the haemocoel
• 15 hpi, after the symbiont has grown in the haemocoel but before the insect dies

And in two organs :
• Midgut (MG), the entrance site of the nematode
• Fat body (FB), the key organ of insects' systemic humoral immune response and a central organ of insects' metabolism

In three independent experiments :
- Polyadenylated RNAs from the organs of nine infested and control last instar larvae were purified and a single-end 50 base pairs Illumina sequencing was performed
- Sequences were pseudo-aligned on the *S. frugiperda* reference transcriptome OGS2.2 using the Kallisto software
- Differential gene expression was determined with the Sleuth software (Wald test, $q\text{-value} < 0,01, |\beta| > 1$)
- Tissue-specific responses were explored using the gene ontology software Blast2GO, manual annotation and the R package gplots.

Results

Venn diagram of specific and shared differentially expressed genes in each organ :



Overall differential expression

- At 8 hpi :**
- ❖ No transcriptional response in the midgut
 - ❖ Weak transcriptional response in the fat body
- At 15 hpi :**
- ❖ Strong transcriptional response in both organs
 - ❖ Shared transcripts between the two organs are mainly involved in developmental processes and protein translation

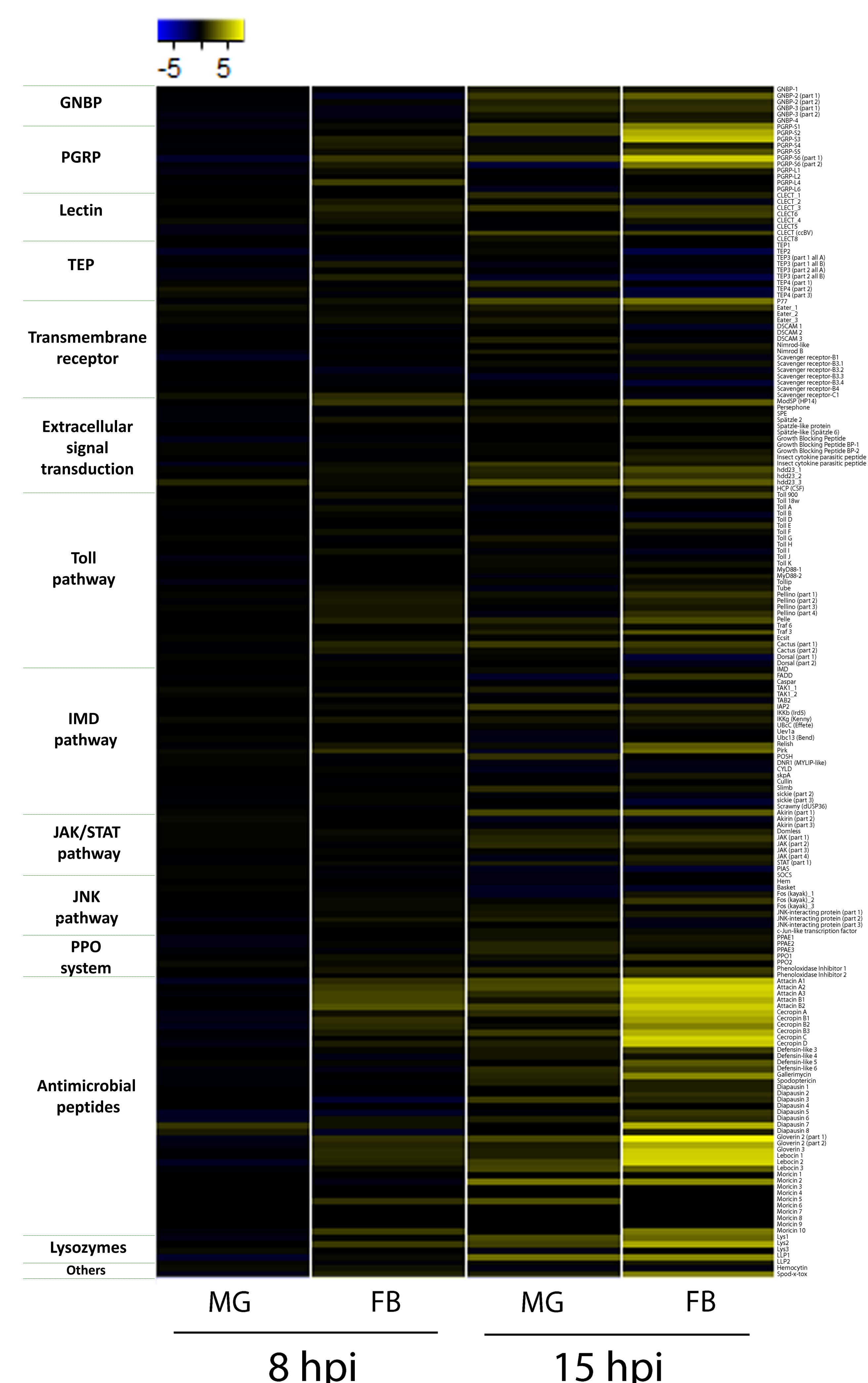
GO term enrichment of fat body-specific overexpressed genes at 15 hpi (Fisher's exact test, FDR = 0,05)

Enriched GO term	% FB-specific GO terms	% Reference GO terms
Innate immune réponse	6,99%	0,27%
Defense réponse to bacterium	5,51%	0,19%
Extracellular region	11,40%	2,82%

Focus on humoral immune response

- At 8 hpi :**
- ❖ No immune response in the midgut
 - ❖ Weak immune response in the fat body
- At 15 hpi :**
- ❖ The fat body produces a strong immune response dominated by the overexpression of attacin, cecropin, gloverin and leboicin antimicrobial peptides and of some short PGRPs. The Toll pathway seems to be activated whereas the IMD pathway seems to be inhibited.
 - ❖ The midgut produces a slight immune response, characterized by the overexpression of a different set of antimicrobial peptides.

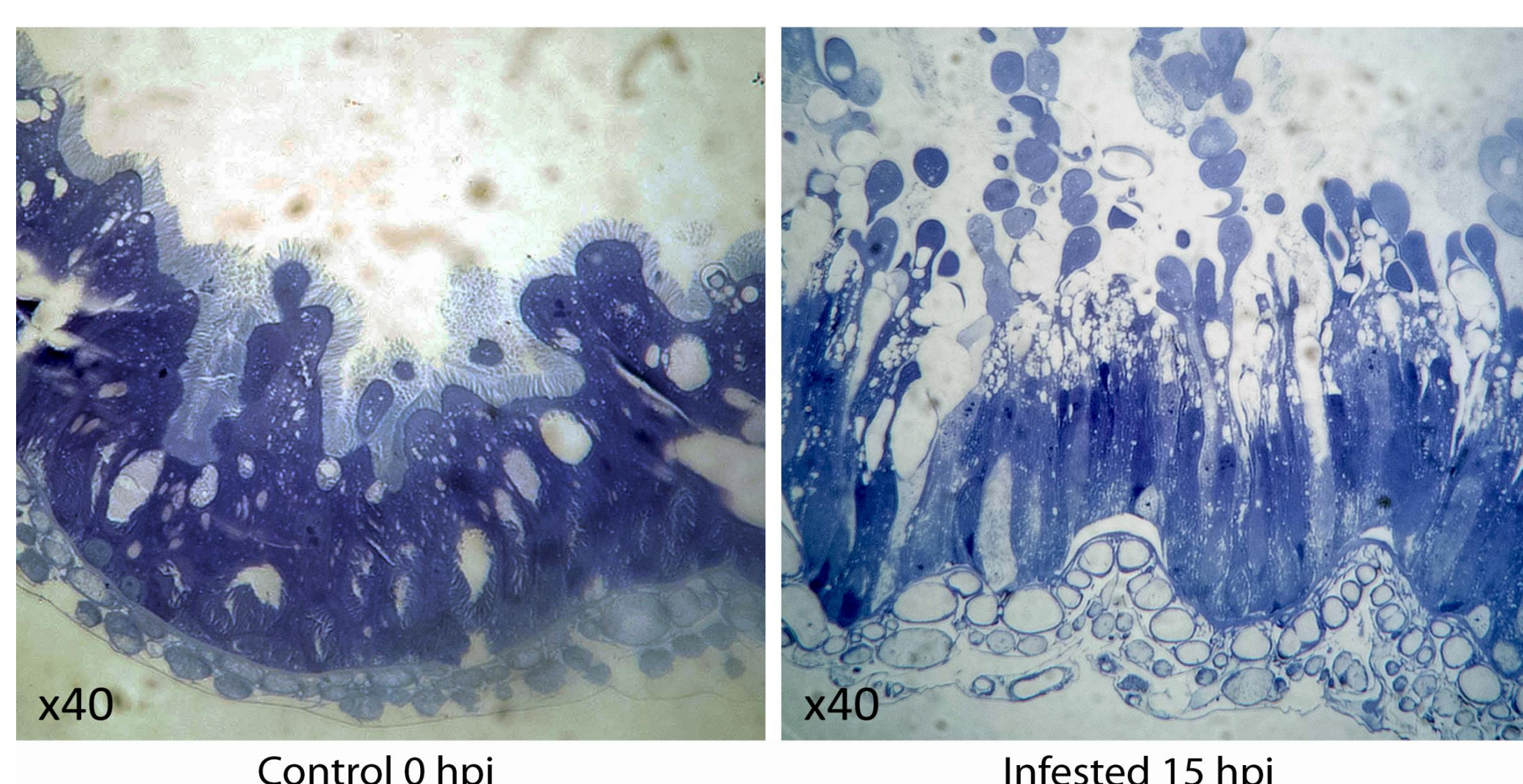
Heatmap showing differential expression of the *Spodoptera frugiperda* humoral immune genes :



Tissue-specific responses

- Fat body :**
- ❖ Overexpressed genes are mainly involved in immunity
- Midgut :**
- ❖ The response is not enriched in any functional category, but some of the most overexpressed genes are involved in detoxification and autophagy
 - ❖ The epithelium seems to undergo cell death at 15 hpi only

Transversal cuts of the midgut epithelium of control and infested larvae at 15 hpi :



Discussion

- Preliminary experiments indicate that some nematodes are found in the intestinal tract as early as 4 hpi and that the first *X. nematophila* are found in the hemolymph at 6 hpi. Thus, the absence of transcriptional response in the midgut at 8 hpi could indicate that the nematode and its passing through the intestinal barrier are undetected in this organ. This is in agreement with the normal aspect of the midgut epithelium at 8 hpi.
- On the other hand, in the fat body, a slight immune response is produced at 8 hpi and is strongly amplified at 15 hpi. Our results seem to indicate an antibacterial immune response.
- At 15 hpi, the midgut overexpresses detoxification genes and produces a slight immune response, but its aspect suggests that it is undergoing enterocyte apoptosis. Toxins that are produced by both *S. carpocapsae* and *X. nematophila* could be responsible of this cell death phenomenon.
- Finally, this analysis raises questions about the *Spodoptera frugiperda*'s immune response. Indeed, our results indicate that the Toll pathway might be activated and the Imd pathway inhibited. This may be surprising because in model insects, the Imd pathway is preferentially activated against Gram negative bacteria such as *X. nematophila* and this pathway induces the synthesis of antimicrobial peptides such as attacins whose genes are all strongly upregulated in our study.