Biologie Adaptative des Pucerons
et Organismes Associés


Acyrthosiphon pisum © INRA, Bernard Chaubet

Aphids Special Interest Group
Royal Entomological Society

UK – French joint meeting on Aphids

5-6 November 2015 – Paris (France)

Programme

Thursday 5th November

9h00 – 10h00 Registration (with tea/coffee)

10h00 – 10h10 Welcome and introduction

Chair: Federica Calevro

10h10 – 10h50 Invited presentation

Takema Fukatsu *(National Institute of Advanced Industrial Science and Technology - Japan)*

Symbiosis, evolution and biodiversity

10h50 – 11h15 2 x 12.5 min talks

Colin Turnbull *(Imperial College London - UK)*

Genetics of virulence in pea aphid. *[Turnbull C., Kanvil S., Selby M., Powell G.]*

Steve Foster *(Rothamsted Research - UK)*

Insecticide resistance: the attack of the clones. *[Foster S., Malloch G., Fenton B., Williamson M.]*

11h15 – 11h45 Refreshment break

Chair: Richard Harrington

11h45 – 12h50 5 x 12.5 min talks

Sam Mugford *(John Innes Centre - UK)*

Discovery of effector proteins that modulate plant-insect interactions in the genome of *Myzus persicae*. *[Mugford S., Drurey C., Chen Y., Mathers T., Van Oosterhout C., Swarbreck D., Hogenhout S.]*

Grit Kunert *(Max-Planck Institute for Chemical Ecology - Germany)*

Who is manipulating whom? - The relationship between pea aphid biotypes and their host plants. *[Kunert G.]*

Jens Joschinski *(University of Würzburg - Germany)*

Day length constraints and diurnal activity of the pea aphid. *[Joschinski J., Krauss J.]*

Freddy Tjallingii *(EPG systems - Netherlands)*

Early sieve element interactions during stylet penetration. *[Tjallingii W.F.]*

Thomas Mathers *(The Genome Analysis Centre - UK)*

Evidence for ancient expansions of gene families in aphids and genome streamlining in *M. persicae*. *[Mathers T., Chen Y., Mugford S., Van Oosterhout C., Swarbreck D., Hogenhout S.]*

12h50 – 14h00 Lunch

Chair: Marylène Poirié

14h00 – 15h15 6 x 12.5 min talks

Yazhou Chen *(John Innes Centre - UK)*

Transcription plasticity of specific gene families are involved in *Myzus persicae* adaptation to diverse plant species. *[Chen Y., Mathers T., Mugford S., Van Oosterhout C., Swarbreck D., Hogenhout S.]*

Hugh Loxdale *(University of Cardiff - UK)*

Ghost of the clones. *[Loxdale H.D.]*

Martin Drucker *(INRA BGPI - France)*

TuMV aphid transmission activation. *[Drucker M.]*

Craig Webster *(INRA BGPI - France)*

Stylin-1, the first protein located in the acrostyle and its role in non-circulative virus transmission by aphids. *[Webster C., Hoh, F., Van Munster M., Gargani D., Padilla A., Bron P., Blanc S., Uzest M.]*

Alexandra Schoeny *(INRA Pathologie Végétale - France)*

Relationship between *Aphis gossypii* population dynamics and Cucurbit aphid-borne yellows virus (CABYV) epidemics in melon crops. *[Schoeny A., Gognalons P., Wipf-Scheibel C., Millot P., Nozeran K., Lecoq H.]*

Lise Roy *(Université de Montpellier - CEFE - France)*

Dynamics of insecticide resistance alleles in the green peach-potato aphid *Myzus persicae*. *[Roy L., Barrès B., Capderrey C., Rohrlich C., Fontaine S., Micoud A., Simon J-C.]*

15h15 – 15h45 Refreshment break

Chair: Simon Leather

15h45 – 17h00 6 x 12.5 min talks

David Hopkins *(University of Sheffield - UK)*

The chemical ecology of divergent host plant acceptance behaviour in the speciation in the pea aphid (*Acyrthosiphon pisum*). *[Hopkins D., Butlin R., Cameron D.]*

Christoph Vorburger *(ETH Zurich and Eawag - Switzerland)*

Bacterial endosymbionts protect aphids in the field and alter parasitoid community composition. *[Vorburger C., Rothacher L.]*

Marylène Poirié *(Institut Sophia Agrobiotech - France)*

“Modulation” of the pea aphid immune defense: influence of the genotype, developmental stage and symbionts. *[Villalba J., Schmitz A., Tares S., Gatti J-L., Poirié M.]*

Ryuichi Koga *(National Institute of Advanced Industrial Science and Technology - Japan)*

Making the world colorful with FISH. *[Koga R.]*

Gaël Le Trionnaire *(INRA IGEPP - France)*

From non-coding RNAs to epigenetics: integrative genomics to understand reproductive polyphenism re-gulation in the pea aphid. *[Le Trionnaire G., Legeai F., Wucher V., Richard G., Leterme-Prunier N., Jaquiéry J., Jaubert-Possamai S., Hudaverdian S., Tanguy S., Nicolas J., Seitz H., Derrien T., Gallot A., Tagu D.]*

Charles Dedryver *(INRA IGEPP - France)*

From the XVII to the XX century, the main steps of understanding aphid biology. *[Dedryver C-A., Pierre J-S.]*

17h00 – 18h30 Poster session *(see below the list of posters)*

18h45 – 22h00 Conference dinner

Friday 6th November

Chair: Christoph Vorburger

9h15 – 10h05 4 x 12.5 min talks

Emilie Bosquée *(Gembloux Agro-Bio Tech – Université de Liège - Belgium)*

Multitrophic interactions between plants-viruses-aphidiphagous. *[Bosquée E., Lopes T., Boullis A., Heuskin S., Sarles L., Fassotte B., Lognay G., Verheggen F., Francis F.]*

Claire Drurey *(John Innes Centre - UK)*

The *Myzus persicae* effector Mp10 suppresses ROS and Ca^{2+} bursts and modulates PAMP-triggered immunity. *[Drurey C., Prince D., Zipfel C., Hogenhout S.]*

Samir Fakhour *(ELIB - Université Catholique de Louvain - Belgium)*

Diversity and geographic distribution of major cereal aphids' bacterial communities in Morocco. *[Fakhour S., Ambroise J., Foray V., Hance T.]*

Thomas Vincent *(John Innes Centre - UK)*

Defending plants against the World's most pesticide-resistant insect, *Myzus persicae*: a role for calcium. *[Vincent T., Mugford S., Pitino M., Toyota M., Gilroy S., Miller T., Hogenhout S., Sanders D.]*

10h05 – 10h35 Refreshment break

Chair: Saskia Hogenhout

10h35 – 11h25 4 x 12.5 min talks

Emmanuelle Jousselin *(INRA-CBGP - France)*

Role of host-plant adaptation and symbiont associations in the diversification of a globally distributed aphid genus (Lachninae: Cinara). *[Jousselin E., Meseguer S.A., Clamens A-L., Coeur d'Acier A.]*

Frederic Francis *(Gembloux Agro-Bio Tech – Université de Liège - Belgium)*

How to control aphid: basic to applied multidisciplinary approaches, from lab to field. *[Francis F.]*

Mélanie Leclair *(INRA IGEPP - France)*

Phenotype diversity in pea aphids carrying the *Hamiltonella defensa* bacteria. *[Leclair M., Pons I., Mahéo F., Van Gheluwe C., Toussain G., Gauthier J-P., Denis G., Le Gallic J-F., Simon J-C., Outreman Y.]*

Antoine Boullis *(UEFE - Gembloux Agro-bio Tech - Belgium)*

Impact of atmospheric change in CO2 concentration on aphid escape behavior. *[Boullis A., Appeldoorn C., Oostrom M., Francis F., Verheggen F.]*

Co-chairs: Jean-Christophe Simon & Denis Tagu

11h25 – 12h15 Plenary discussion

12h15 – 13h30 Lunch

Chair: Jim Hardie

13h30 – 14h45 6 x 12.5 min talks

Véronique Brault *(INRA SVQV - France)*

A synthetic aphid virus for gene functional validation. *[Clavijo G., Rustenholz C., Calevro F., Ogliastro M., Simon J-C., Monsion B., Boissinot S., Mulot M., Bochet N., Reinbold C., Brault V.]*

Enric Frago *(Laboratory of Entomology, Wageningen University - Netherlands)*

Bacterial symbionts mediate complex interactions between aphids and parasitic wasps. *[Frago E., Mala M., Godfray H.C., Dicke M., Gols R.]*

Ailsa McLean *(University of Oxford - UK)*

Effects of endosymbionts on natural enemy resistance in pea aphids. *[McLean A., Hrček J., Parker B., Godfray C.]*

Chris Bass *(Rothamsted Research - UK)*

Metabolic resistance to natural and synthetic xenobiotics in the aphid, *Myzus persicae*. *[Bass C.]*

Stéphane Blanc *(INRA BGPI - France)*

A plant virus is a long-life potion for aphid vectors. *[Yvon M., Michalakis Y., Blanc S.]*

Quentin Chesnais *(Université de Picardie - BIPE - France)*

Convergence of aphid manipulation by phytoviruses according to their mode of transmission: is it always the case? *[Chesnais Q., Couty A., Uzest M., Brault V., Ameline A.]*

14h45 – 15h15 Refreshment break

Chair: Véronique Brault

15h15 – 16h30 6 x 12.5 min talks

Arnaud Sentis *(Université de Toulouse - ENFA - France)*

Impacts of warming, trophic interaction and interclonal competition on life history traits, phenotypic plasticity, and population dynamics of a pink and a green clone of the pea aphid. *[Sentis A., Kondratow F., Achkar T., Danchin E., Hemptinne J-L.]*

Pierre Simonet *(INRA/INSA-Lyon BF2I - France)*

Direct flow cytometry measurements and cell imaging reveal a fine-tuning of endosymbiont and bacteriocyte cell dynamics during aphid development. *[Simonet P., Duport G., Gaget K., Balmand S., Colella S., Febvay G., Charles H., Heddi A., Calevro F.]*

James Bell *(Rothamsted Research - UK)*

Climate change impacts on aphid migration. *[Bell J.R., Henrys P.A, Thackeray S.J., Harrington, R.]*

Julien Bauwens *(University of Liege - Gembloux Agro-Bio Tech - Belgium)*

Impact of *Regiella insecticola* infection on EBF production in *Acyrthosiphon pisum* Harris. *[Bauwens J., Mazzuchelli G., De Pauw E., Tsuchida T., Francis F.]*

Alejandro Manzano-Marin *(University of Valencia - Spain)*

A tale of two symbionts: diversity of secondary co-obligate symbionts in the Lachninae subfamily. *[Manzano-Marín A., Szabo G., Simon J-C., Horn M., Latorre A.]*

Larissa Collins *(Fera - UK)*

In-field aphid monitoring and virus transmission in UK crops. *[Collins L.]*

16h30 – 16h45 Conclusions and depart

Posters

Sanaa Abed *(Swansea University - UK)*
Biodiversity of natural enemies and the control of agricultural pests. *[Abed S.N., Griffin J.N., Fowler M.S.]*

Gia Aradottir *(Rothamsted Research - UK)*
Dreams and reality: searching for wheat resistance to cereal aphids. *[Aradottir G., Martin J., Pickett J., Clark S., Smart L.]*

Refka Ben Issa *(INRA - PSH - France)*
Effect of intercropping pepper with companion plants on the settling of aphid population under greenhouses conditions. *[Ben Issa R., Gomez L., Gautier H.]*

Florent Bogaert *(Université de Picardie - BIPE - France)*
How nitrogen fertilization may influence crop resistance to aphids : the case of *Miscanthus*, a promissing biomass crop . *[Bogaert F., Chesnais Q., Doury G., Catterou M., Rambaud C., Dubois F., Ameline A.]*

Aude Couty *(Université de Picardie - BIPE - France)*
Effects of a phytovirus on the foraging behaviour and life history traits of an aphid parasitoid. *[Moiroux J., Chesnais Q., Verrier E., Ameline A., Couty A.]*

Tarek Dardouri *(INRA PSH - France)*
Behavioral response of green peach aphid to volatiles from different rosemary clones. *[Dardouri T.]*

Emyr Davies *(Rothamsted Research - UK)*
An evolutionarily-unique heterodimeric voltage-gated cation channel found in aphids. *[Amey J., Burton M., O'Reilly A., Puinean M., Grzeskowiak J., Williamson M., Mellor I., Duce I., Field L., Wallace B., Davies T.G.E.]*

Damian De Marzo *(Fera - UK)*
The highs and lows of yellow water trap aphid monitoring. *[De Marzo D.]*

Anastase Harelimana *(ELIB - Université Catholique de Louvain - Belgium)*
Varietal screening based on demographic parameters: resistance of coffee to *Toxoptera aurantii*. *[Harelimana A., Hance T., Rukazambuga D., Legoff G., Migon M.]*

Heïdi Kaech *(Eawag - Switzerland)*
Symbiont-conferred resistance compromises aphid control by parasitoids. *[Kaech H., Vorburger C.]*

Rachel Kehoe *(University of Exeter - UK)*
Experimental evidence for the population-dynamic mechanisms underlying extinction cascades of carnivores. *[Sanders D., Kehoe R., van Veen F.]*

Thomas Lopes *(Gembloux Agro-Bio Tech - ULg - Belgium)*
Species diversity of aphids and their natural enemies in crop associations. *[Lopes T., Hatt S., Starý P., Japoshvili G., Francis F.]*

Hugo Mathé-Hubert *(Eawag - Switzerland)*
Defensive symbiosis in aphids – linking within host mechanisms with ecology and evolution. *[Mathé-Hubert H., Kaech H., Vorburger C.]*

Michaël Mulot *(INRA Colmar - France)*
Intestinal membrane proteins of *Myzus persicae* involved in polerovirus transmission. *[Mulot M., Boissinot S., Rastegar M., Monsion B., Brault V.]*

Ferran Palero *(INRA-PACA - France)*
Metagenomic anaysis of *Macrolophus pygmaeus* (Hemiptera: Miridae). *[Palero F.]*

Valentin Pierson *(ELIB - Université Catholique de Louvain - Belgium)*
Exploring the place of *Serratia symbiotica* in the symbiotic interaction between ants and aphids. *[Pierson V., Renoz F., Foray V., Hance T.]*

Inès Pons Guillard *(ELIB - Université Catholique de Louvain - Belgium)*

Costs, benefits and immune defences in the relationship between insects and symbiotic bacteria. *[Pons I., Renoz F., Hance T.]*

François Renoz *(ELIB - Université Catholique de Louvain - Belgium)*

A comprehensive proteome map of an insect symbiont for better understanding the mechanisms of symbiosis. *[Renoz F., Champagne A., Degand H., Faber A-M., Foray V., Hance T.]*

Gautier Richard *(INRA IGEPP - France)*

Genome scale identification of open chromatin regions in the pea aphid. *[Richard G., Legeai F., Leterme-Prunier N., Jaquiéry J., Le Trionnaire G., Tagu D.]*

Aurélien Sallé *(LBLGC – Université d'Orléans - France)*

Gall-inducing aphid depletes the reserves of its host tree. *[Sallé A., Jerger R., Vincent-Barbaroux C., Bourgerie S., Lieutier F.]*

Laurent Serteyn *(Gembloux Agro-Bio Tech – Université de Liège - Belgium)*

Hemipteran - host plant interactions: focus on some model insects saliva. *[Serteyn L., Moreau S., Delatour A., Francis F.]*

Akiko Sugio *(INRA IGEPP - France)*

Plant-aphid-symbiont interactions: what are the factors that maintain their association? *[Sugio A., Morlière S., Guy E., Boulain H., Jousseaume T., Le Gallic J-F., Aigu Y., Schaedel K., Rispe C., Nouhaud P., Legeai F., Outreman Y., White F., Simon J-C.]*

Maria Zhang *(University of Reading - UK)*

The influence of climate on autumn UK abundance of the *Rhopalosiphum padi*. *[Zhang H., Harrington R., Breeze T., Bailey A., Potts S.]*

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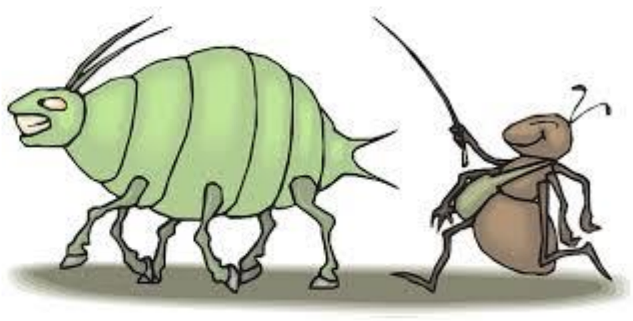
Talks



Symbiosis, evolution and biodiversity

Takema Fukatsu
National Institute of Advanced Industrial Science and Technology (AIST), Japan

Many insects and other organisms constantly harbor microorganisms inside their body. Due to the close spatial proximity, extremely intimate biological interactions and inter-dependency are commonly found between the partners called host and symbiont. Novel biological properties are often generated through such associations. In many cases, host and symbiont are integrated into an almost inseparable biological entity.

For a long time, the pea aphid *Acyrthosiphon pisum* and the obligatory bacterial symbiont *Buchnera* and also the facultative bacterial associates *Hamiltonella*, *Serratia*, *Regiella* etc. have provided a model system of the insect-microbe symbiotic association. Meanwhile, bacterial symbionts are ubiquitously found in diverse insects, from which new excellent model systems are emerging recently.

Here I introduce such new model systems for insect symbiosis studies, by which some important problems that are difficult to address with the aphid system can be beautifully solved.

Genetics of virulence in pea aphid

Colin Turnbull*, Sadia Kanvil, Martin Selby, Glen Powell
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Many aphids display strong host choices and vary greatly in success on different hosts, but we know relatively little of the genetic basis of compatible and incompatible interactions. At the molecular level, effectors from the aphid are injected via saliva into the host, acting as virulence factors that may suppress host immunity and/or facilitate feeding. Alternatively, if the host carries a resistance (*R*) gene that recognises one or more incoming effector type, then it may counteract the virulence functions, resulting in classification of the aphid as avirulent on that host. Here, we focus on the so-called gene-for-gene interactions in the pea aphid – *Medicago truncatula* model system. We show that virulence of a range of aphid clones on a panel of *M. truncatula* genotypes varies greatly in a genotype x genotype manner. Mendelian segregation for virulence on genotypes carrying a resistance QTL was found in F_1 and backcross populations derived from crosses and self-matings of avirulent and virulent clones. We interpret these data to indicate that much of the variation in virulence is accounted for by only a few genes. In progeny from self-mating of an avirulent parent we unexpectedly found gain-of-virulence phenotypes, suggesting that recombination even within a single population can rapidly lead to novel resistance-breaking genotypes. We conclude that controlling genotypes of both aphid and host provides a route towards definition of cognate effector – R gene relationships.

Insecticide resistance: the attack of the clones

Steve Foster[1], Gaynor Malloch[2], Brian Fenton[3]and Martin Williamson[1]
[1]Rothamsted Research, England
[2]James Hutton Institute, Scotland
[3]SRUC, Scotland

Biochemical and molecular genetic diagnostics have provided an unparalleled understanding of the incidence and impact of different insecticide resistance mechanisms, especially in the Peach-potato aphid, *Myzus persicae*. It is clear in this species that, as a consequence of prolonged parthenogenesis in field populations, combinations of resistance mechanisms have become 'locked together' in 'super-clones' so that they piggy-back, even when the insecticides corresponding to the resistance mechanisms are no longer used. Microsatellite analysis has shown that two such super-clones (O and P), in *M. persicae*, and one super-clone clone (SA3), in *Sitobion avenae*, have come to predominate recently in the UK. These clones carry insecticide resistance mechanisms that have appeared in genetic backgrounds that appear to be well adapted for survival in the UK, particularly during cold or wet winters such as those seen in 2012/13 and 2013/14 respectively.

Discovery of effector proteins that modulate plant-insect interactions in the genome of the green peach aphid.

Sam T. Mugford[1], Claire Drurey[1], Yazhou Chen[1], Tom Mathers[2], Cock Van Oosterhout[3], David Swarbreck[2] and Saskia Hogenhout[1].
(sam.mugford@jic.ac.uk)
[1]Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK;
[2]The Genome Analysis Centre, Norwich Research Park, Norwich, NR4 7UH, UK;
[3]School of Environmental Sciences University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ

The green peach aphid (GPA) *Myzus persicae* is an important crop pest. GPA is unusual amongst herbivorous insects in being polyphagous; it is able to colonize and feed from plants belonging to at least 40 different families. We hypothesize that the remarkable plasticity of GPA lies in its ability to suppress a wide variety of plant defence mechanisms, and that this is enabled –at least in part- by effector proteins capable of modulated host defenses that GPA delivers into the plant when feeding. We sequenced the genome of GPA, and compared to that of the specialist pea aphid *Acyrthosiphon pisum*, which in contrast to GPA mostly colonizes a single plant family. We mined the GPA genome for candidate effector proteins, based on a range of criteria including expression in the salivary gland, possession of predicted secretory signal peptides, differential expression on different host plants, and signs of positive selection. From this set of candidate effector proteins, we selected some for the generation of antibodies, which were used to demonstrate expression in aphids and presence in plant tissue at the site of aphid feeding. We also show that knock-down of the expression of candidate effectors in aphids affects the defense response of plants to those aphids, in particular the PAMP-triggered immune response pathway, and impairs the ability of the aphids to survive and reproduce. Ongoing work aims to characterize the targets and mode of action of these proteins.

Who is manipulating whom? – The relationship between aphids and plants.

Grit Kunert
Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany

Herbivorous insects are the most diverse group of multicellular organisms inhabiting our world. One process that may have contributed to this enormous biodiversity is ecological speciation via continuous host plant switches. We use the pea aphid (*Acyrthosiphon pisum*) complex as a model system to study this process. The pea aphid complex consist of at least 15 genetically distinct and sympatric host races which are adapted to specific host plants of the Legume family, but can all develop on the universal host plant *Vicia faba*. However, the role of the host plant defense and chemistry for this adaptation has not been studied so far. Thus we investigated how certain host and non-host plants react to feeding attempts of different pea aphid host races. Phytohormone analyses revealed that plants react differently depending on the aphid race which was infesting the plant. By using a mass spectrometry-based non-targeted metabolomic approach we observed not only a change of the plant metabolome upon aphid infestation but also a metabolome change depending whether an adapted or a non-adapted aphid race was feeding on the plant.

Day length constraints and diurnal activity of the pea aphid

Jens Joschinski[1*], Jochen Krauss[1]
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Climate change can alter the phenology of organisms. It may thus lead seasonal organisms to face different day lengths than in the past, and the fitness consequences of this novel time budget are as yet unclear.
The pea aphid *Acyrthosiphon pisum* generally induces sexual morphs under short days, but some clones have lost this phenological trait secondarily. Because these obligately asexual clones can be used to study day length effects without eliciting a seasonal response, we recorded life-history traits of one such clone under short and long days and under two realistic temperature cycles with means differing by 2°C.
We show that short days reduce fecundity and the length of the reproductive period of aphids. Nevertheless this does not translate to differences at the population level, because the observed fitness costs only become apparent late in the individual's life. Hence fecundity is a poor predictor of aphid fitness.
To provide a mechanistic explanation of the observed costs of short days, we tested whether aphids are day-active animals, and how the circadian clock is organised. During day-time *A.pisum* moulted more frequently and produced more honeydew. Artificial diets are promising tools to study aphid clocks, as they allow separating aphid and host rhythms. At the moment several shortcomings of artificial diets stall research on aphid clocks, but we provide solutions to overcome some of the methodological difficulties. We think that research on aphid clocks help understanding the true complexities of time measuring in nature.

Early sieve element interactions during stylet penetration

Freddy Tjallingii
EPG Systems, Wageningen, Netherlands

Two distinct EPG waveforms are shown upon sieve element (SE) puncturing by aphid stylets: 1) waveform E1, related to watery salivation and subsequently 2) waveform E2, related to ingestion *and* concurrent watery salivation. The E1 saliva is injected into the SE. The E2 saliva does not reach the SE as it is mixed with the SE sap and forced into stylet food canal after opening the cibarial valve. The function of E1 and E2 salivation has been attributed to prevention or reversion SE wound responses - due to clogging or expansion of phloem (P-) proteins - leading to occlusion of SEs and the aphid's food canal, respectively. Calcium binding was supposed the main mechanism in preventing forisomes (a P-protein body in fabaceae) plugging as shown by *in vitro* experiments using collected aphid saliva and isolated forisomes. However, recent *in vivo* evidence showed that in compatible pea aphid/*Vicia* combination no forisome expansion occurs after a stylet puncture. Also, when forisome plugs were evoked by remote plant damage, aphids switching then from E2 to E1 salivation did not revert back forisomes to a non-plugging state any faster than forisomes in not punctured neighbouring SEs. After a SE stylet puncture, E1 salivation normally starts in about 10s. But forisome plugging in non-host plants was shown to start before E1. From EPGs it seems likely that early pd-like salivation may be responsible for occlusion in non-hosts or avoiding occlusion on hosts. Whether Ca ion channels or other Ca ion sources may be involved will be discussed.

Evidence for ancient expansions of gene families in aphids and genome streamlining in *M. persicae*.

Thomas Mathers[1], Yazhou Chen[2], David Swarbreck[1], Cock Van Oosterhout[3], Sam Mugford[2] and Saskia Hogenhout[2]
(thomas.mathers@TGAC.ac.uk)
[1]The Genome Analysis Centre, Norwich Research Park, Norwich, NR4 7UH, UK; [2]Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK; [3]School of Environmental Sciences University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UK.

The green peach aphid *Myzus persicae* is a major worldwide agricultural pest. Unlike the majority of plant feeding insects, which tend to specialise on a single plant species or family, *M. persicae* is able to colonise over 400 plant species from a diverse range of families. Coupled with its ability to reproduce asexually and widespread insecticide resistance, this makes *M. persicae* a highly effective plant pest. To investigate the evolution of generalism and the mechanisms behind host plasticity in *M. persicae* we and collaborators at INRA-Rennes (Denis Tagu and Fabrice Legeai), University of Miami (Alex Wilson) and Boyce Thompson Institute (Georg Jander) sequenced the genomes of two clones, the widespread UK clone O and an American clone, G006. Comparative analysis of *M. persicae* gene families with the pea aphid *Acyrthosiphon pisum* and 19 other arthropod species revealed a dynamic pattern of gene family evolution in aphids. We observed a high rate of gene gain and loss in both aphid species relative to other insects, with many aphid specific gene duplication events having occurred. However, contrary to expectations, the evolution of generalism in *M. persicae* has not been accompanied by widespread gene family expansions specific to this lineage. Rather, we see reduced retention of duplicate genes and stronger purifying selection compared to the specialist aphid *A. pisum*, consistent with genome streamlining and the discriminate preservation of functional genes in *M. persicae*.

Specific gene families are involved in *Myzus persicae* response to diverse plant species

Yazhou Chen[1], Thomas Mathers[2], Sam Mugford[1], Cock Van Oosterhout[3], David Swarbreck[2], Saskia Hogenhout[1]
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The green peach aphid *Myzus persicae* is a major worldwide agricultural pest. Unlike the majority of plant feeding insects, which tend to specialise on a single plant species or family, *M. persicae* is able to colonise over 400 plant species from a diverse range of families. Coupled with its ability to reproduce asexually and widespread insecticide resistance, this makes *M. persicae* a highly effective plant pest. To better understand how *M. persicae* can colonize diverse plant species, we employed RNA-seq to profile gene expression patterns of clonal lineages of *M. persicae* clone O reared on Chinese cabbage (*Brassica rapa*), *Arabidopsis thaliana* and *Nicotiana benthamiana*. In total, 171 genes were differentially expressed (DE) between the aphid lineages. These included genes for peptidases, cuticular proteins and proteins involved in protein modification, transcription, transport and metabolism. Interestingly, 10 cathepsin B (peptidase) genes and 23 cuticular protein genes are arranged in clusters of tandemly repeated units in the *M. persicae* genome and were DE in the opposite directions depending on the plant species. We conducted host transfer experiments between *A. thaliana* and *N. benthamiana* and showed that the expression levels of the cathepsin B and cuticular protein genes adjusted within only two days upon plant host switch of individual aphids. Typically, cathepsin B genes were upregulated in aphids on *B. rapa* and *A. thaliana*, whereas the cuticular protein genes were upregulated in those on *N. benthamiana*. Knocking down the expression levels of cathepsin B family genes by plant-mediated RNA interference (RNAi) reduced aphid reproduction on *A. thaliana*. Taken together, these data indicate tight regulatory control of the tandemly repeated genes and the involvement of cathepsin B genes in aphid-plant interactions.

Ghost of the Clones

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In 1922, John C. Willis, FRS (1868-1958), described the so-called Willis hollow curve, in which the *Number of genera* is plotted against the *Number of species per genus*, giving for many species tested, both animal and plant, a negative exponential curve. Since then, theoretical discussion continues to revolve around the meaning of such curves. In about 2009, I plotted aphid microsatellite genotypes as the *Frequency of Occurrence* vs. *Number of sets of different MLGs* (multilocus genotypes). I got clear negative exponential curves for two tansy-feeding aphid species (*Macrosiphoniella tanacetaria* and *Metopeurum fuscoviride*), and also for a cereal aphid species (*Sitobion avenae*), this sampled by hand in the field and captured using 12.2. m high suction traps. The last result suggested that the results obtained were not artefactual, i.e. resulting from sampling error. The graph showed that most aphid MLGs occurred as low copy numbers (1 = `unique', 2, 3 times, etc., i.e. `specialised genotypes') and that few showed high copy numbers (say > 10 times, i.e. `generalist genotypes'). Unbeknown to me at the time I had, some 90 years later, re-invented a version of the Willis hollow curve, but for microsatellite MLGs rather than species within genera. Here I discuss what this may mean in terms of the specialism vs. generalism debate and how the curve may reflect the 'ghost of the clones', i.e. those asexual genotypes that ultimately adaptively radiate and become new sub-specific or even specific forms in their own right.

Turnip mosaic virus is a second virus that responds to the presence of aphid vectors and activates transmission

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Turnip mosaic virus (TuMV) is transmitted by aphids and using, like hundreds of other plant virus, the noncirculative mode, according to the molecular “helper” strategy. This indicates that the virus particles (virions) transmitted attach within seconds to the stylets and are transported to a new host plant. The connection between TuMV and the stylets is not direct, a transmission helper component (the viral protein HC-Pro Helper Component Protease) intervenes by creating the molecular link between virions and stylets. For Cauliflower mosaic virus, another noncirculative virus using the helper strategy, but entirely unrelated to TuMV, the presence of vectors on the plant induces the passage from a state where the virus is not transmissible to a state where it becomes transmissible (Bak et al. 2013; Martinière et al. 2013). The transmission of TuMV has been tested in order to determine if this virus also follows this strategy, called "Transmission Activation (TA)." Aphid transmission tests were performed using infected protoplasts as viral source, which were incubated or not with different reagents. The results of these tests showed that a ROS (Reactive Oxygen Species), H2O2, activates the transmission of TuMV while a blocker of calcium signaling, LaCl3, inhibits it. Then, by the technique of SDS-PAGE/Western blot, a phenotype for TuMV TA could be characterized by the presence / absence of oligomeric HC-Pro in infected cells, which correlated with the activation and inhibition of the transmission of TuMV by aphids. Thus, TuMV is a second example for the TA.

Stylin-1, the first protein located in the acrostyle and its role in non-circulative virus transmission by aphids

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While the receptors of *Cauliflower mosaic virus* (CaMV) have been located at the tip of maxillary stylets within the acrostyle, an organ likely bearing the receptors of all non-circulative phytoviruses transmitted by aphids, the identity of virus receptors remains unknown. Here we identify for the first time a cuticular protein that is accessible at the surface of the acrostyle in maxillary stylets. This protein, Stylin-1, is present at the stylet tip of common vectors of CaMV. A series of specific antibodies allowed the mapping of one of the Stylin-1 peptide domains readily accessible at the surface, thus at the interface with external compounds. Reactions of Stylin-1 to specific enzymatic treatments of dissected stylets are consistent with those of CaMV receptors. Preliminary competition/blocking experiments for acrostyle binding indicate that the CaMV ligand protein P2 significantly reduced labelling by @Stylin-1 antibodies. This result identifies, for the first time, a specific cuticular protein present in the stylets of aphids and which is part of the acrostyle. Furthermore the role of this protein as the first receptor of a non-persistent virus is discussed.

Relationship between *Aphis gossypii* population dynamics and *Cucurbit aphid-borne yellows virus* (CABYV) epidemics in melon crops

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The temporal dynamics of alate aphids and viruses were established in eight field trials conducted between 2010 and 2014. Alate aphids visiting melon crops were sampled daily using succion traps and frequently encountered aphid species (*Aphis craccivora*, *Aphis fabae*, *Aphis gossypii*, *Myzus persicae*) were identified. Persistant *Cucurbit aphid-borne yellows virus* (CABYV) and non-persistent *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) were monitored weekly by DAS-ELISA. Gompertz model was fitted to each incidence data set by nonlinear regression and AUDPCs (Area Under the Disease Progress Curve) were calculated. A statistical analysis was performed to explore the relationships existing between several “aphid” variables (cumulative abundances over different periods of time, per species or globally) and several “virus” variables (cumulative numbers of infected plants over different periods of time, newly infected plants per week, AUDPCs, disease onset day, Gompertz model parameter estimates). No relationship was highlighted between aphids and non-persistent viruses. More interestingly a predictive relationship was established between *Aphis gossypii* population dynamics and CABYV epidemics. Indeed, the number of *Aphis gossypii* trapped between the 14 first days of the crop explained more than 50% of the variability of the CABYV total AUDPC. This result suggests that controlling precociously the population of *Aphis gossypii* could impact favourably the epidemic onset and progress of this virus in melon crops.

Dynamics of insecticide resistance alleles in the green peach-potato aphid *Myzus persicae*.

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Insecticides remain the main control strategy against crop pests. Due to different histories of insecticide applications between crops, heterogeneous selective pressures may be exerted on pest populations and lead to develop different insecticide resistance mechanisms in agricultural landscapes. Depending on the dispersal capacity and the fitness on the different host species, polyphagous resistant genotypes may spread among different crops. Here we analysed the spatio-temporal dynamics of target resistances in the peach-potato green aphid over a French multi-crop region. The genetic structure was characterized at different spatial and temporal scales, using both neutral (microsatellites) and selected (insecticide target proteins) molecular markers. Very limited gene flow was observed between primary (peach on which sexual reproduction takes place) and secondary (herbaceous) hosts. Non synonymous mutations in the cDNA of pyrethroid, carbamate and neonicotinoid targets were widespread, with frequency distribution strongly varying between the two types of hosts. All dominant clones were recurrently found on different secondary host species and possessed target resistances. This suggests that the most successful resistant genotypes on secondary hosts are generalist. Consequently, early emergence of resistance against newly authorized molecules on one secondary host may occur by contagion from long exposed other secondary hosts. The replacement of a dominant clone by another one was shown to be completed within 2-3 years. Present data allowed to trace the emergence in 2004, subsequent increase and wide-scale colonization of a resistotype combining mutant targets of both pyrethroids and carbamates and associated to two dominant clones.

The chemical ecology of host plant speciation in the Pea Aphid (*Acyrthosiphon pisum*)

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The huge diversity of phytophagous insects is largely attributable to speciation involving shifts between host plants. These shifts are mediated by the close interaction between insects and plant metabolites, which may act as feeding stimulants or repellents, or influence insect performance, for example through toxicity. However, there has been only limited progress in understanding the chemical signatures that underlie insect preferences. Here, we use the pea aphid (*Acyrthosiphon pisum*) to address this question. Host-associated races of pea aphid discriminate between plant species and some of their chemosensory genes appear to have diverged under selection. We have combined untargeted metabolomic profiling of multiple plant species of *Medicago* and *Trifolium* with tests of differential acceptance by two *A. pisum* races, using random forest regression to identify metabolites that explain variation in acceptance. Our results reveal that a small number of compounds explain a large proportion of variation in the differential acceptability of plants to *A. pisum* specialised on *Medicago sativa* or *Trifolium pratense*. The identity of some of these compounds was confirmed using tandem mass spectrometry.

Bacterial endosymbionts protect aphids in the field and alter parasitoid community composition

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Many symbionts are passed on from parent to offspring. Vertical transmission couples host and symbiont fitness and has thus facilitated the evolution of defense mechanisms by which symbionts protect their hosts against parasites. A well-known example is the bacterial endosymbiont *Hamiltonella defensa*, which protects its aphid hosts against parasitoid wasps. Laboratory experiments have shown that *H. defensa* does not protect equally well against different species of parasitoids, and that there is among-strain variation with some strains protecting specifically against certain parasitoid species, or even against particular genotypes of the same species. Thus, there is a solid body of laboratory work predicting that defensive endosymbionts play an important role in reducing aphid mortality by parasitoids and presumably affect the community composition of parasitoids that rely on aphids as a resource. However, there is still a conspicuous lack of work showing that this is indeed the case under natural conditions. We tried to close this gap with a field experiment on black bean aphids (*Aphis fabae*). We set up replicated field plots with genetically identical aphids that did or did not harbor *H. defensa* and followed their colonization by natural enemies over a growing season. We observed a clear reduction in parasitism of symbiont-protected aphids, particularly by the parasitoids posing the highest risk, as well as strong shifts in the parasitoid species composition. Despite their reduced parasitism, protected aphids did not develop larger populations than unprotected ones, possibly reflecting the balancing effect of costs associated with harboring *H. defensa*.

“Modulation” of the pea aphid immune defense: influence of the genotype, developmental stage and symbionts

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The field of microbial symbiosis has achieved astonishing advances but the interplay between symbiotic bacteria and the host immunity largely remains unexplored. New insights can be expected from the aphid model that hosts several facultative endosymbionts, in addition to the obligate *Buchnera* bacteria. It is generally believed that the pea aphid model *Acyrthosiphon pisum* has a weak immune response since no “classical” insect antibacterial peptides were identified and the genome lacks key immune genes (e.g. *imd*). Having characterized *A. pisum* immune cells and PO activity, we nevertheless evidenced a functional cellular immune response against free-living bacteria (*E. coli*) as well as secondary symbionts (SS). We then questioned whether host immune components (e.g. PO activity, number of adherent cells) were affected by the presence of different SS (*Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica)*, using aphid lines of the same genetic background but with different SS species/strains. The tested variables “host genetic background” and “artificial/natural infection” had none or little effect while the SS species and strain strongly influenced *A. pisum* aphid components (Schmitz et al., 2012; Villalba et al., submitted). We then followed-up the number of adherent immune cells and PO activity along the aphid development and “identified” the stage at which changes occur in aphids harbouring impacting-SS. A transcriptomic analysis of adherent immune cells of aphids hosting different SS species/strains is under analysis that will hopefully provide insights into the immune pathways activated in response to various SS and explain the phenotypic effects some SS have on host immunity.

Making the world colorful with FISH

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Molecular biological technologies, such as PCR & cloning, Next Generation Sequencing and quantitative PCR, have illuminated and unveiled the species diversity, internal densities and population dynamics of the microbes colonizing the insect body, and contributed to broaden our understanding about the richness of the microbial world exist in insects. To take another step into this world to know natures of the relationship between the host insect and microbial residences, it is very important to locate the microbes in their host body. Where are they living in the host body? Do they show any tissue tropisms? How dense do they colonize? How are multiple microbial species associated? How are they transmitted to offspring? Only histological techniques can provide precise answers for these questions. Fluorescence in situ hybridization with oligonucleotide probes targeting ribosomal RNA of bacteria (FISH) is the most powerful and convenient histological technique for studying the symbiotic relationships between insects and microbes. In this presentation, I will briefly summarize the technical aspects of FISH technique and show some examples of the insect symbiotic systems visualized and colored using this technique.

From non-coding RNAs to epigenetics: integrative genomics to understand reproductive polyphenism regulation in the pea aphid.

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Aphids are hemipterous insects that can cause important damages to a wide spectrum of crops. Their success as pests is largely explained by their remarkable ability to adapt to their biotic and abiotic environment within the agro-ecosystems they evolve in. Phenotypic plasticity is one striking example of such an adaptive potential. It corresponds to the fact that one given genotype can give rise to different and discrete phenotypes in response to the fluctuations of environmental factors. Reproductive polyphenism is one case of plasticity that typically occurs during aphid life cycle. Aphids indeed reproduce quickly and efficiently by parthenogenesis (clonal multiplication) during spring and summer, generating most of the crop damages. At the end of the summer, aphid can perceive the photoperiod shortening. This signal is then transduced towards their ovarioles where embryos fate is changed. Sexual individuals, males and oviparous females are then produced, mate and lay cold resistant eggs that can overcome winter. We've been trying to understand in the last few years the cellular and molecular mechanisms that govern the asexual to sexual embryogenesis switch. We try to understand this phenomenon at the different levels of genome expression regulation, typically from epigenetics to post-transcriptomic events. We've thus developed integrative genomics approaches based on high-throughput sequencing data generation - including RNA-seq, small RNAs-seq and soon FAIRE-seq (for differentially chromatin open regions mapping) data - to establish a dynamic and global view of genome expression control associated with the establishment of alternative phenotypes.

A short story of the beginning of aphidology

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When entomology exists since more than 2000 years, due to their small size, aphids interested entomologists only after appearance of strongly magnifying glasses and microscopes. They have been mentioned firstly in the middle of 17th century, mostly as preys for syrphid larvae, and later in this century have been evidenced the main original features of their biology (viviparity, sap feeding, polyphenism). During the 18th century they became a fascinating material of study for several well-known scientists, because of their different reproductive modes. At this period, they were regarded as an exception in 'the great order of nature' and a case against excessive generalization in biology. Their obvious reproduction without mating caused numerous interrogations. The term 'parthenogenesis' was only created and used in the middle of 19th century, after histological studies on several organism, among them aphids.

Multitrophic interactions between plants-viruses-aphidiphagous

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The Potato Virus Y (PVY) and Potato Leaf Roll Virus (PLRV), transmitted by *Myzus persicae* (Sulzer), can cause serious damages to Solanaceae. It is known that virus-infected plants may affect the behaviour of aphid vectors. Indeed, volatile organic compounds (VOCs) emitted from PLRV-infected potato plants can enhance *M. persicae* attraction, arrestment and promote sustained feeding for virus acquisition. This strategy will ultimately benefit the virus, by increasing its chances of spreading. If VOC from virus-infected plants was demonstrated to attract aphids, what is about the aphidophagous beneficials? To address this question, we compared the olfactory response of the predator *Harmonia axyridis* (Pallas) and parasitoid *Aphidius colemani* Viereck between PLRV or PVY-infected and virus-free tobacco plants (*Nicotiana tabacum* Linnaeus). VOCs emitted from different treatments were collected and identified by GC-MS. *H. axyridis* and *A. colemani* adults were significantly more attracted by odours emitted from infected compared with uninfected plants. Qualitative and quantitative differences were observed between VOCs emitted from treatments. This study contributes to better characterize interactions between virus-infected plants and natural enemies of vectors.

Aphid attack triggers a plant defence response involving PAMP-triggered immunity pathways and aphids can suppress this defence using effectors

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Aphids are insects, which feed on phloem sap using their stylets. This can lead to significant losses of crop yield, both through the drain of plant resources and the vectoring of plant viruses. Due to these negative effects, it would be expected that plants have developed defenses against them. In plant-pathogen interactions, basal plant defense involving pathogen associated molecular pattern (PAMP) triggered immunity (PTI) effectively fends off the majority of plant pathogens. We have found that aphids can evoke typical PTI defense responses such as reactive oxygen species (ROS) bursts and callose deposition. Successful colonization of the host plant by an aphid is thought to involve overcoming this defense response. Suppression of the plant immune response is suggested to be carried out using effectors, which are most likely salivary gland proteins introduced into the plant during aphid feeding. Previously a salivary gland protein from the aphid *Myzus persicae,* Mp10, was identified which suppresses the (ROS) burst elicited by the PAMP flg22 (Bos, Prince *et al.,* 2010. PLoS Genetics 6(11): e1001216). Further investigation of Mp10 function revealed that it also suppresses the calcium burst that precedes the flg22 ROS burst, as well as the ROS burst elicited by crude aphid extract. Silencing experiments show that Mp10 is needed for aphid success on the plant, as measured by fecundity. In conclusion, our results so far indicate that PTI plays a role in plant defense response to aphid attack and is suppressed by an aphid effector.

Diversity and geographic distribution of major cereal aphids' bacterial communities in Morocco.

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Associations with microorganisms are ubiquitous in insects. Through improved resource exploitation or resistance to environmental stresses, they have allowed insects to colonize environments to which they would not otherwise have access. By fulfilling an essential nutritional function, some associated bacteria are obligate partners for their host, while others are labeled as facultative symbionts by being only beneficial in the context of various ecological conditions. Many scientific progresses have been made in the description of aphid symbiont diversity, but how bacterial communities evolve according to environmental factors remains poorly understood. Here, we analyzed the microbial diversity of five cereal aphid species sampled on three different host plants for a total of 131 populations. The bacterial diversity has been characterized by 16S rRNA genes sequencing with high-throughput sequencing instruments, and the geographical distribution of bacterial infections according to several environmental factors has been investigated. Sequencing yielded 98,500 bacterial 16S rRNA reads on average per sample, and 17 operational taxonomic units (OTUs) were detected, representing eight bacterial genera. The microbiome of all samples was dominated by obligate and facultative symbionts previously described in aphids. The overall bacterial diversity depends on aphid species, altitude and bio-climate. Two significant associations were confirmed: *Regiella insecticola* was found in *Sitobion avenae* with a prevalence of 84%, and *Serratia symbiotica* was found in *Sipha maydis* with a prevalence of 100%. This study is an essential step for understanding occurrence and functional dimensions of the biodiversity of cereal aphid-associated bacteria.

Key words : bacterial diversity, geographical distribution, Cereal aphids, symbionts, 16S rRNA amplicon, Morocco.

Calcium signalling in plant-aphid interactions

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Myzus persicae (the green peach aphid) holds the Guinness World Record as the most resistant insect to pesticides, documented to being resistant to 71 different varieties. *M. persicae* also acts as a vector for over 100 plant viruses, many of which have devastating effects on crop production. It is distributed across the globe, successfully colonising every continent except Antarctica, and has an enormous host plant range of over 70 families.

Part of *M. persicae's* success is down to its ability to suppress the plant defence response. A pivotal part of this response is hypothesised to be calcium signalling. Using the latest *in vivo* calcium sensors, we have been able to track and characterise these signals at the point of aphid attack with high spatial and temporal resolution.

These signals are pronounced at the feeding site once the aphid settles on a leaf, but also travel systemically in the plant vasculature. One gene we have implicated in this response is Two-pore Channel 1 (TPC1), a plant vacuolar calcium channel. TPC1 is known to be a key mediator of long-distance calcium signals in plants, and our work further cements the vital role of this gene in defence signalling. Knocking-out TPC1 significantly reduces aphid-induced calcium signals. Furthermore, through electro-penetration graph (EPG), fecundity and plant biochemical assays, we have established altered aphid performance on TPC1 mutants, as well as altered plant defences in these lines.

Role of host-plant adaptation and symbiont associations in the diversification of a globally distributed aphid genus

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Cinara is the second most diversified aphid genus and one of the few genera known to feed on conifers. Most species are generally host-specific and this genus is considered to be a recently divergent lineage originating from a rapid radiation following the acquisition of a gymnosperm host. It also shows repeated intercontinental disjunctions in the Holarctic suggesting that geographic isolation might account for major diversification events in this genus. Finally, several studies suggest that the diversification of the genus has been accompanied by major changes in the composition and metabolic capabilities of their bacterial endosymbionts, with *Serratia symbiotica* fulfilling some essential metabolic functions that are no longer ensured by *Buchnera aphidicola* in several species. These characteristics make Cinara a good model ideal to investigate the relative importance of biotic (i.e. host-plants & endosymbionts) and abiotic factors (i.e. climate & geography) in the diversification of aphids. In order to address this question, we 1) reconstruct phylogenetic relationships in *Cinara* using six DNA fragments for 372 specimens representing 92 out of 248 *Cinara* spp (spanning the biogeographic and ecological diversity of the genus); 2) investigate the biogeographic history of the genus and the evolution of host-association, 3) characterize the community of endosymbiotic bacteria associated with our sampling using deep sequencing of 16S rRNA bacterial genes and infer how changes in this community has accompanied major evolutionary transitions in *Cinara*.

How to control aphid: basic to applied multidisciplinary approaches, from lab to field.

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Several sustainable strategies to manage pests such as aphids are frequently cited to promote biological control. At laboratory scale, different approaches can de developed to investigate aphid – plant interactions : (1) direct defences and subsequent adaptations in both kinds by developing « omics » techniques to identify the active compounds from plant, the dynamic responses of aphids in terms of physiological and metabolic adaptations ; (2) indirect defences focusing on semiochemicals and their role in tritrophic interactions either as attractive or repellent toward aphid and/or aphidophagous beneficials by developing electrophysiological and behavioural assays. Moreover, each kinds of protagonist, plant and aphid, should not be considered alone but associated to their related microbiomes. After more fundamental laboratory experiments, field applications come as a logical step to go further to functional development. Field management for plant diversity, application of infochemical slow releasers are promoted and applied in different crop systems to increase push-pull strategy to control aphid. According to different aphid – plant – predator model investigations, the importance of multidisciplinary approaches and strategies will be discussed to allow both fundamental understanding and applied new development for aphid control.

Phenotype diversity in pea aphids carrying the *Hamiltonella defensa* bacteria

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A majority of species interacts, closely and durably, with symbiotic microorganisms. These latter can modify the ecology and evolution of their hosts by inducing a variety of effects on their phenotype. However, the extended phenotype associated with a symbiotic organism can vary largely depending on the host species. For example, the symbiont *Wolbachia* can manipulate the reproduction of its host, protect it against natural enemies or enhance its nutrition. All these effects induced by *Wolbachia*, which can be deleterious or beneficial, depend on the host species.

The aim of our study is to measure the variability of phenotypes that can be associated with a bacterial symbiont within a single host species. Our biological model is the pea aphid (*Acyrthosiphon pisum*). This particular aphid presents populations that have specialized on different legumes species (ie, biotypes). Among the symbiotic bacteria hosted by this insect, *Hamiltonella defensa* is highly prevalent in different biotypes but its phenotypic effects – altered behavior, reduced fitness and parasitic resistance - are known only in two of these biotypes. By analyzing the life history traits and the level of resistance of individuals carrying this symbiotic bacterium from four *A. pisum* biotypes, our study reveals that there is a high variability associated with this bacterium amongst inter and intrabiotype phenotypes. Therefore, a symbiotic microorganism/eukaryote relationship cannot be summarized by a single phenotypic effect.

Impact of atmospheric change in CO_2 concentration on aphid escape behavior

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The effects of global atmospheric changes on vegetation and associated insect populations are increasingly studied, but how these changes affect the interactions between herbivorous insects and their natural enemies is less clear.

Aphids produce an alarm pheromone to signal the presence of a natural enemy in the colony and to induce conspecifics to escape. Here, we test the hypothesis that changes in CO_2 atmospheric concentration affects the chemical communication established between aphids and their natural enemies.

Firstly, we quantified the production as well as the emission of (*E*)-β-Farnesene (*E*βF - main compound of alarm pheromone) released by predated aphids, reared under ambient ($aCO_2 \approx 400$ ppm) or elevated CO_2 ($eCO_2 \approx 800$ ppm) concentration. Using dynamic headspace volatile collection, we found no difference between aCO_2 and eCO_2 aphids for both, production of *E*βF and its emission by predated aphids. Contrariwise, using a real-time analyzer gas chromatograph (zNose) which allowed us to record the emission of *E*βF by predated aphids, we found that eCO_2 aphids emit more *E*βF than aCO_2 aphids.

Secondly, we look at the influence of CO_2 concentration on alarm pheromone perception by aphids and their associated escape behavior. When synthetic *E*βF was presented to an aphid colony, the proportion of aphids leaving the test area is not different between the two populations (aCO_2 & eCO_2).

These results will be discussed in terms of biological control in future climate.

A synthetic aphid virus for gene functional validation

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Gene functional validation in aphids can be conducted by several approaches based on RNA interference (RNAi) mechanism. The strategy relies on the micro-injection or oral acquisition of double-stranded RNA (dsRNA) priming the RNAi mechanism. In the present study, we developed a new functional validation tool in which *Myzus persicae densovirus* (MpDNV) is used as a gene vector that can potentially reduce or overexpress candidate genes. MpDNV is a non-enveloped, single-stranded DNA virus which, in contrast to other densoviruses, has only a minor effect on the fitness of *M. persicae*. This virus was observed by electron microscopy in anterior midgut cells and is horizontally and vertically transmitted to the progeny. MpDNV is highly prevalent in natural *M. persicae* populations and integration of densovirus-like sequences into *M. persicae* genome could be responsible for its harmlessness.

A high throughput sequencing approach was conducted to obtain the complete MpDNV sequence and more particularly the sequence of the missing highly structured inverted terminal repeats at the 3’ and 5’ ends. A synthetic MpDNV vector containing the full-length MpDNV sequence modified by the introduction of several restriction sites was further constructed. A first experiment was performed in which the MpDNV vector was delivered orally or by micro-injection to an aphid clone deprived of free MpDNV. 20% of the aphid progeny showed the presence of the viral DNA. This encouraging result, which needs to be further confirmed, may lead to an innovative tool to validate the function of *M. persicae*’s genes.

Bacterial symbionts mediate complex interactions between aphids and parasitic wasps

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It has long been known that insects are colonised by a diverse community of mutualistic symbionts that provide their hosts with nutrients that are missing in the diet. In the last decade, it has become apparent that facultative symbionts (i.e. not required for host survival) in herbivore insects can also play an important role in insect-plant interactions. When attacked by insects, plants are able to defend themselves by triggering defensive mechanisms aimed at poisoning the herbivore or at attracting its natural enemies. Recent research demonstrates that facultative insect symbionts can play an important role in this context, for example by down-regulating the expression of plant defensive traits. We explore the role of pea aphid (*Acyrthosiphon pisum*) facultative endosymbionts in plant defences. Our results suggest that insect symbionts can have intricate effects on aphid natural enemies, some of them mediated by changes in plant quality.

Effects of endosymbionts on natural enemy resistance in pea aphids

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Many aphids harbour facultative endosymbiotic bacteria, some of which have been shown to provide resistance against natural enemies. One of the best-known protective endosymbionts is *Hamiltonella defensa*, which appears to defend its host aphid from parasitoid wasps by preventing the successful development of their eggs and larvae. We have found that the degree of protection observed varies considerably between different bacterial strains and against different parasitoid species. This raises the question of why aphids do not routinely maintain multiple symbiont infections to allow broad-spectrum protection. We are starting to answer this question experimentally by transferring multiple species or strains of bacteria via microinjection into single aphid individuals and examining the infection patterns and survival outcomes that result.

Metabolic resistance to natural and synthetic xenobiotics in the aphid, *Myzus persicae*

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The peach potato aphid, *Myzus persicae* is the most economically important aphid pest worldwide. This species is globally distributed and highly polyphagous with a host range of over 400 species including many important crop plants. The intensive use of insecticides to control this species over many years has led to populations that are resistant to a remarkable number of different compounds. A major focus of our work is to understand, at the molecular level, how *M. persicae* detoxifies natural and synthetic xenobiotics such as insecticides and the anti-herbivore defence chemicals produced by many plants. In this talk I will highlight recent examples of our work on this topic and the evolutionary insights it has provided.

A plant virus is a long-life potion for aphid vectors.

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In the last ten years, the study of diverse plant-virus-vector systems has consistently demonstrated that viruses can manipulate their insect vectors in a way that increases transmission. This manipulation is shown to possibly operate through two distinct mechanisms: i) a direct effect of the virus within its insect vector and/or ii) an indirect effect via physiological changes induced by the virus in the infected host plant. In all cases, the analyzed parameters are the attractiveness of the plants for insect vectors depending on whether these plants are infected or healthy, the settlement of aphids onto infected compared to healthy plants, and growth rate of the insect populations estimated through generation time and number of offspring. It is remarkable that all parameters analyzed are when the insect vector is on the host plant, and that the phase when it is “off plant” is seldom considered. Yet, for a transmitted virus, the capacity of the vector to successfully find another host plant, and the distance or surface area the vector can explore are obviously key for survival and epidemiology.

We have decided to explore whether a virus (FBNSV, *Nanoviridae*) within an infected plant can turn an aphid (*A. pisum*) into a “super-aphid” when leaving the infected plant and searching for another one. We targeted the “mobility” and the length of survival time as two putatively important aphid properties, which could be manipulated by the virus. While mobility may represent an enhanced exploratory behavior and increase the chance of quickly finding new host plants, surviving longer without food and drinks would also increase the chance of finding a new host and the distance at which this host can be found. We have very preliminary data showing that the virus can significantly change both traits, and we start accumulating reproducible evidence of long-surviving aphids after a few days of feeding on infected plants. Though preliminary, these results show that individual performances of a vector when travelling off host plants can be extremely important and are likely targeted for manipulation by viruses. These aspects have been vastly ignored thus far and should be investigated in other plant-virus-vector systems.

Convergence of aphid manipulation by phytoviruses according to their mode of transmission: is it always the case?

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Vector-borne viruses can induce changes in the phenotypes of their host plants that influence the frequency and nature of host–vector interactions. Insect-vectored phytoviruses that differ in their modes of transmission (persistent, semi-persistent and non-persistent) benefit from different patterns of interaction among host plants and vectors. Manipulation of vector behaviour by phytovirus facilitate its propagation and seems to converge according to the virus transmission mode. However, this convergence has barely been studied in viruses infecting Brassicaceae.

The present study has tested the effects of infection of *Camelina sativa* with the persistent *Turnip Yellow Virus* and the semi-persistent *Cauliflower Mosaic Virus* on (i) arrestment and dispersal, (ii) feeding behaviour by electropenetrography and (iii) physiology of two aphid species: the polyphagous *Myzus persicae* and the Brassicaceae specialist *Brevicoryne brassicae*.

Results showed no evidence of evolutionary convergence in vector behaviour within persistent and within semi-persistent phytovirus-infected camelina. Indeed, whatever the virus species considered, infected camelina had opposite effects (*i.e.* negative and positive) on the feeding behaviour and physiology of the two aphid species.

In conclusion, viral manipulation of vector behaviour could therefore depend not only on the virus mode of transmission, which is generally considered as a common assumption, but also on the aphid species; indeed, aphid species with different host range differed in their response to the same virus infection.

Key words: *TuYV*, *CaMV*, green peach aphid, cabbage aphid, false flax, EPG, physiology, vector manipulation, virology.

Impacts of warming, trophic interaction and interclonal competition on life history traits, phenotypic plasticity, and population dynamics of a pink and a green clone of the pea aphid.

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Revealing the links between life history trait variations and species interactions is of paramount importance for understanding and predicting the impacts of climate change on the dynamics and stability of ecological communities. However, little is known about the interactive effects of environmental perturbations on individual species, intra- and interspecific interactions and trait-mediated effects. Using a plant-aphid-ladybeetle system, we experimentally investigated the effects of warming, trophic interaction, and interclonal competition on life history traits, phenotypic plasticity (wing morph induction by predators), and demography of two pea aphid clones. We found that warming tends to increase aphid population growth rates but this effect was only significant for one clone in absence of predators. Interestingly, predation and warming respectively increased and decreased the proportion of winged aphids. Finally, interclonal competition increased aphid population growth rates and aphid body mass whereas it decreased the proportion of winged aphids. Furthermore, the magnitude of these effects depended on the clones, temperature, and presence of predators. Our study reveals that complex interactions between abiotic and biotic factors affect variations in aphid life history traits, population dynamics and phenotypic plasticity. We conclude that considering environmental factors, intraspecific variability and species interactions is crucial to improve our ability to predict the impacts of global changes on individual species and the diversity and stability of ecosystems.

Key words: climate change, interclonal competition, trait-mediated effects, polyphenism, trophic interactions, pea aphid, ladybeetles.

Direct flow cytometry measurements and cell imaging reveal a fine-tuning of endosymbiont and bacteriocyte cell dynamics during aphid development

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Endosymbiotic associations constitute a driving force in the ecological and evolutionary diversification of metazoan organisms. Little is known about whether and how symbiotic cells are coordinated according to host physiology. Here, we use the nutritional symbiosis between the insect pest, *Acyrthosiphon pisum*, and its obligate symbiont, *Buchnera aphidicola*, as a model system. We have developed a novel approach for unculturable bacteria, based on flow cytometry, and used this method to estimate the absolute numbers of symbionts at key stages of aphid life. The endosymbiont population increases exponentially throughout nymphal development, showing a growing rate which has never been characterized by indirect molecular techniques. Using histology and imaging techniques, we have shown that the endosymbiont-bearing cells (bacteriocytes) increase significantly in number and size during the nymphal development, and clustering in the insect abdomen. Once adulthood is reached and the laying period has begun, the dynamics of symbiont and host cells is reversed: the number of endosymbionts decreases and the bacteriocyte structure progressively degenerates during insect aging. To characterize the retrogressive changes in bacteriocyte tissue, *in situ* hybridization and immunohistochemistry experiments have been performed and demonstrated the appearance of disaggregated bacteriocyte cells exhibiting morphological alterations such as low symbiont-density zones and abnormal nuclear shape. Altogether, these results show a coordination of the cellular dynamics between bacteriocytes and primary endosymbionts and reveal a fine-tuning of aphid symbiotic cells to the nutritional demand imposed by the host physiology throughout development.

Climate change impacts on aphid migration

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Recently, large-scale phenological changes (i.e. the seasonal timing of biological events) in marine, freshwater and terrestrial environments have been linked to climate change. A meta-analyses has been conducted which demonstrated that aphids are showing some of the fastest responses to climate change.

We studied the role of temperature and precipitation on the leading edge of the population (i.e. first flight and fifth percentile of flight) using a two-stage analytical process. First, data were processed to detect a climate sensitivity window, matching phenology with 5x5 km gridded daily air temperature and precipitation data. Climate sensitivity profiles (CSPs) were generated to express the change in seasonal timing per unit change in temperature (days $^{\circ}C^{-1}$) or precipitation (days mm^{-1}). These CSPs identified key seasonal periods within which temperature and precipitation were most, and least, influential. All CSPs were then aggregated and an overview was generated using a global linear mixed-effect model. The data matrix included 55 aphid species across 17 sites in the UK between the years of 1965-2010. In total 22,448 site-year-species records were analysed.

Only 0.1% of all precipitation analyses were shown to be statistically linked to any type of phenological change. The link between aphids and temperature, particularly winter temperature, has repeatedly shown to be statistically strong. This also proved to be the case in our analysis: 72.6% of time series were statistically significant for producing earlier phenologies with warmer temperatures. Rarely did warmer temperatures produce statistically significant later phenologies (0.2%). A low developmental threshold temperature, a high intrinsic rate of increase along with plastic life history traits (e.g. facultative anholocycly) ensure that aphids are well placed to benefit from the global challenges that undoubtedly lie ahead.

Impact of *Regiella insecticola* infection on EBF production in *Acyrthosiphon pisum* Harris.

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Symbionts of aphids influence their host from many points of view. We investigate the potential influence of bacterial symbionts on the production and emission of the aphid alarm pheromone, E-β-franesene. Some trends could be observed in the total EβF production. Particularly, aphid strains infected by Buchnera only seemed to produce less alarm pheromone. By contrast, the presence of Regiella insecticola seemed to increase EβF production. Mevalonate pathway was investigated by RT-qPCR. This analysis showed a slightly lower transcription level o mIPPS in Regiella-infected strains. This enzyme is involved in the last step of EβF production. By contrast, two enzymes involved respectively in the linkage and release of farnesyl moeities on proteins c-terminal ends. Escape tests were conducted to assay if these results were traduced by differential behavior in front of a predator. Preliminary results showed significantly higher dropping behavior for Regiella-infected strains.

A tale of two symbionts: diversity of secondary co-obligate symbionts in the Lachninae subfamily.

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Most aphids harbour the obligate endosymbiotic bacteria *Buchnera aphidicola.* These, endow the aphids with an expanded set of metabolic capabilities such as biosynthesis of essential amino acids and some vitamins which are required for their survival. In the Lachninae subfamily it was found that many members seemed to be always associated to a *Serratia symbiotica* secondary endosymbiont. Through whole genome sequencing, we have determined that *Cinara tujafilina* (Eulachnini), *Cinara cedri* (Eulachnini) and *Tuberolachnus salignus* (Lachnini) have indeed established co-obligate associations with the typical *B. aphidicola* plus *S. symbiotica*, given a common loss of the riboflavin biosynthetic pathway by *Buchnera*. Nevertheless, while in the former, *S. symbiotica* is extracellular, rod shape, and possesses a big genome, in *C. cedri* and *T. salignus* the *S. symbiotica* endosymbionts are intracellular, pleomorphic and have very small genomes that have apparently arose independently. However, some aphids such as *Maculolachnus submacula* (Lachnini) and *Eulachnus rileyi* (Eulachnini) have been found to be associated to other bacterial taxa, rather than *S. symbiotica*. We have analysed representatives from the different genera of the Lachninae with both FISH and PCR, and have corroborated that all possess two co-obligate endosymbionts. While for most it is *S. symbiotica*, some members have lost this and replaced it by at least two different bacterial taxa. Therefore, this aphid subfamily could stand as a model for symbiont replacement and settlement, and through further genomic investigation, it is expected to shed light on the settling-down processes and the timing of the different colonization and genome reduction events.

In-field aphid monitoring and virus transmission in UK crops

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Aphid transmitted viruses cause significant yield losses and quality issues in UK crops, including salads, carrots, parsnips, potatoes and oilseed rape. To assist growers in controlling vectors and their associated viruses Fera operates a national Yellow Water Trap Network in seed potato crops. The national network is funded by AHDB. Fera also offers aphid monitoring on a commercial basis to growers of other crops to assist them in making more informed decisions about control of aphid pests and virus vectors. We have also recently re-assessed the potential of some UK aphid species to vector potato viruses. The aphid transmitted viruses Potato virus Y (PVY) and Potato virus A (PVA) are the viruses most commonly affecting seed potatoes in the UK. The Transmission efficiency for each aphid species is used to calculate a potential transmission risk and is expressed as a Relative Efficiency Factor (REF). These REF's have not previously been calculated for UK strains of viruses or UK aphid clones. Using a previously published method, REF's have been calculated for the aphid species and viruses commonly occurring in UK potatoes. This study also represents a first report that *Cavariella aegopodii* has the ability to vector PVY and confirms the potential of *Sitobion avenae*, *Aphis fabae*, *Macrosiphum euphorbiae* and *Rhopalosiphum padi* as important PVY vectors.

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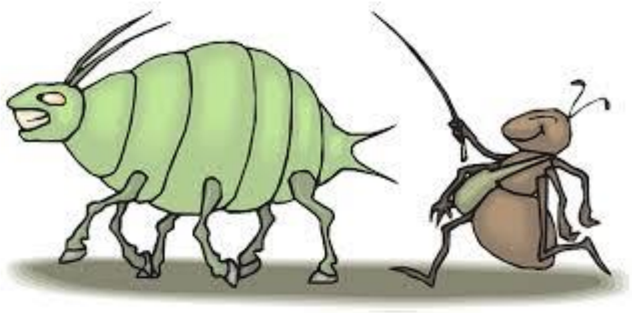

Biodiversity of Natural Enemies and the Control of Agricultural Pests

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Natural enemy biodiversity has an important role in agricultural pest suppression. However, the question of whether the number of enemy species (diversity *per se*) or the particular composition of enemy species is more important in determining pest control, and ultimately crop yield. We tested the effects of Natural enemy diversity and composition (particular mix of species within a given level of species richness) on pea aphids *Acyrthosiphon pisum* and broad bean plants *Vicia faba*. The enemies we used were the larvae of two predator species, the ladybird *Adalia bipunctata* and the green lacewings *Chrysopa carnea*, and parasitic wasps *Aphidius ervi*. The data were analysed with a Nested ANOVA approach, where species composition/ identity was nested within species richness/diversity. We found enemies generally reduced aphid density and indirectly increase the biomass of plants. Among enemy treatments, species richness did not affect aphid density or plant biomass, but the composition of enemies within diversity levels significantly affected both responses. The two-species combination of ladybird and parasitoid performed best among the treatments in aphid control. While plant biomass was improved with each of single predators the lacewings, the ladybird and the combination of ladybird and parasitoid treatments. In conclusion, increasing natural enemy diversity does not consistently affect aphid density or plant biomass. Rather, the combination of certain enemies is more important than species number *per se* in the biological control of aphids.

Dreams and reality: searching for wheat resistance to cereal aphids

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It is of great importance to the resilience of crops that sufficient genetic diversity is retained and recovered from diverse germplasm in order to respond to pressures of abiotic and biotic stresses, including insect feeding. Here we report results of phenotyping efforts to look for resistance to the cereal aphids *Rhopalosiphum padi* and *Sitobion avenae*. Phenotyping was done on hexaploid wheat landraces from the Watkins collection, which originates from the 1930s and covers a near global distribution, as well as cultivars of the Gediflux collection, adapted to the narrow geographic region of north-western Europe and in use between 1945 -2000. Field trials showed collection differences in aphid performance with fewer aphids populating lines from the Watkins collection overall under field conditions. This contrasts with development data acquired in the laboratory bioassays where aphids were confined to the plant using clip cages, and no difference was observed in aphid performance between the Watkins and Gediflux collections. This is interesting and suggests that there is a pre-alighting cue deterring the aphid settlement, which could have important implications for aphid management in wheat production and will enable future studies on the chemistry and genetics involved in susceptibility differences to aphids. It also demonstrates differences in aphid preference and performance on older plants in the field compared to on seedlings in the laboratory, highlighting the need for phenotyping for aphid resistance at different plant growth stages.

Effect of intercropping pepper with companion plants on the settling of aphid population under greenhouses conditions

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Field and laboratory studies support the idea that companion plants may be grown in association with crops for purposes of pest management. Companion plants may affect the settling of pests on their hosts due to their potential to emit volatile organic compounds (VOCs), and their effectiveness depends on the species but also on their arrangement in the crop system. The aim of this study was to evaluate the effect of intercropping companion species (rosemary, lavender or basil) on the settling success of *Myzus persicae* populations on pepper plants (*Capsicum annum*) under greenhouse conditions. The settlement and reproductive performance of females were evaluated for different arrangements of companion plants in the vicinity of pepper plants. In a first set of experiments, pepper plants were neighboured by either one or two companion plants. In another set of experiments, rosemary (the most efficient plant in the first set of experiments) was used to examine the companion plant's scope of action on aphid performance when placed at three distances (0.5; 1.5; 2.5 m) from the host plant. The first set of experiments showed similar patterns of aphid performance when a pepper plant was surrounded by one or two companion plants. However, aphid performance (number of females and nymphs) was more affected when pepper plants were intercropped with rosemary compared to lavender or basil. In addition, the companion plant's effect significantly decreased with distance. Rosemary was most effective at 0.5 m, but its effectiveness decreased at 1.5 m, becoming insignificant at 2.5 m. The results suggest that a companion plant may be effective under greenhouse conditions provided that it is located near the target plant.

How nitrogen may influence crop resistance to aphids: the case of *Miscanthus*, a promising biomass crop.

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The need to substitute fossil fuels to reduce CO2 emissions and to ensure energy security has led to an increasing interest for perennial grasses as alternative sources of energy. Of these plants, *Miscanthus sinensis*, *Miscanthus sacchariflorus* and their triploid sterile hybrid *Miscanthus* x *giganteus* have attracted keen interest as potential biomass crops during the 1990's. These promising candidates are characterized by a high biomass yields, a high tolerance to abiotic stress, a low susceptibility to insect pests and a very low needs in terms of chemical inputs. For example, the nitrogen (N) requirements of these crops are low during the first two years, unless they were planted on poor soils. However, nitrogen fertilization, even at low concentrations, could modify plant resistance against herbivores. Our objective is to evaluate the effect of nitrogen input on the three Miscanthus species (biomass and C/N ratio study) and on its common aphid *Rhopalosiphum maidis* (feeding, weight increase, reproduction and survival study). On *M. sinensis* our results show that nitrogen fertilization (1) enhanced biomass and leaf C/N ratio and (2) affect negatively the feeding behavior and the fitness of aphids. Nitrogen fertilization didn't impact the biomass of *M.* x *giganteus* and *M. sacchariflorus* but decrease the leaf C/N ratio on *M. sacchariflorus*. Moreover, on these two species, the nitrogen fertilization didn't affect aphid's fitness but enhanced its weight. As revealed by the results, contrary to *M. sacchariflorus* and *M.* x *giganteus*, nitrogen fertilization on *M. sinensis* enhance resistance on *R. maidis*. Finally, the consequences of N fertilizer supply on miscanthus were discussed in terms of agronomic implications.

A plant virus affects fitness of an aphid parasitoid, *Aphidius ervi*, but not its foraging behaviour.

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Aphids are major vectors of plant viruses which are responsible for important yield losses. Recently, it has been shown that parasitoids may facilitate plant virus dispersal by disturbing aphids, which move to another plant when attacked. We tested the hypothesis that a plant virus may affect parasitoid attraction by plants and/or aphids to increase its transmission to other plants. We also investigated the aphid-mediated effect of virus on parasitoid fitness. We used the system composed of the host plant *Camelina sativa*, the persistent *Turnip Yellow Virus*, the green peach aphid *Myzus persicae* and the parasitoid *Aphidus ervi*.

To test these hypotheses, we quantified parasitoid attraction towards infected and uninfected plants and measured their attack rate when exposed to viruliferous and/or non-viruliferous aphids. We also compared fitness parameters of parasitoid females reared on viruliferous and non-viruliferous aphids.

Dual-choice laboratory bioassays revealed that parasitoid females did not preferentially orientate towards infected or uninfected plants and aphids, and attacked viruliferous and non-viruliferous aphids at similar rates. These results suggest that the *Turnip Yellow Virus* does not affect foraging behaviour of parasitoids. However we observed decreased body size, egg load and developmental rate in parasitoids reared on viruliferous aphids. This decrease in fitness components may be explained by the lower quality of viruliferous aphids as hosts (e.g. smaller size).

Therefore, the presence of the *Turnip Yellow Virus* in a field may reduce the biological control by aphid parasitoids, which are unable to select uninfected plants or aphids representing better quality hosts and consequently produce fewer progeny.

Behavioral Response of Green Peach Aphid to Volatiles From Different Rosemary Clones

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The green peach aphid (*Myzus persicae* (Sulzer)) is a general pest and potentially dangerous. Intercropping of companion plants such as rosemary (*Rosmarinus officinalis* L.) with horticultural crops (pepper) has been proposed as innovative approach to optimize the struggle against this aphid. The volatile organic compounds (VOCs) emitted by rosemary has been shown to have repulsive effect on this aphid. The objective of this study was to identify among five clones of rosemary, those which can be effective against *M. persicae* and identify their emitted COV. Sixteen compounds were identified by analysis of emitted VOCs by GC-MS; each clone is characterized by a specific profile, showing the existence of chemical variability. The effects of VOCs emitted by different clones of *M. persicae* were monitored using an olfactometer. V. Splindler exhibited the higher repulsive efficiency. The variations in VOCs profiles (emitted by different clones), could partially explain the observed difference of repulsive effects on *M. persicae*. This work is a preliminary study to understand the action of rosemary VOCs on *M. persicae* in an attempt to propose innovative low input cropping systems.

An evolutionarily-unique heterodimeric voltage-gated cation channel in aphids

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We describe the identification in aphids of a unique heterodimeric voltage-gated sodium channel which has an atypical ion selectivity filter (DENS instead of the usual DEKA found in other eukaryotes) and, unusually for insect channels, is highly insensitive to tetrodotoxin. We demonstrate that this channel has most likely arisen by adaptation (gene fission) of an invertebrate ancestral mono(hetero)meric channel. This is the only identifiable voltage-gated sodium channel homologue in the aphid genome(s).The uniqueness of the aphid channel may in the future provide opportunities for the design of novel insecticidal compounds exclusively targeting aphids.

The highs and Lows of Aphid yellow pan water trapping

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Many Aphid species can vector viruses in seed potato crops resulting in potentially high yield loss. Aphids and aphid virus transmission can also be problematic for other vegetable, salad and oilseed rape crops. In seed potatoes the Potato virus Y (PVY) and Potato virus A (PVA) are the most common and of greatest economic importance; even relatively low levels of PVY can reduce seed quality. About 40 Aphid species transmit PVY and PVA with varying degrees of efficiency. Of these the peach-potato aphid (*Myzus persicae*) is the most efficient virus vector.

Fera provides the AHDB funded national yellow water trap Aphid monitoring programme in seed potato crops. Trap results are emailed directly to agronomists and uploaded to the Aphid monitoring website providing growers with up-to-date region specific data. This information can help assess the risk of the spread of PVY and PVA in the crop by identifying the species of aphids present. The financial benefits are better target aphicide applications saving money and time and enabling growers to provide customers with full records of Integrated Pest Management methods. Potential environmental benefits are a reduction in the risk of aphid populations developing aphicide resistance and an increase in natural predator levels. Fera also commercially offers Aphid monitoring services to growers of other vegetable and salad crops and Aphid and Cabbage stem flea beetle monitoring in oilseed rape crops.

Attractiveness of coffee varieties to the citrus black aphids *(Toxoptera aurantii*)

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In Rwanda, most of foreign currency comes from the coffee exportation. *Coffee arabica* is the host of many insects including the black citrus aphids *Toxoptera aurantii*. Considerable yield reduction appears when severe and prolonged attacks occur. *T. aurantii* is reported to be responsible for the transmission of pathogens to coffee plants. The main objective of this work is to evaluate the aphids' attractiveness of different commercial coffee varieties. To achieve our objective; varieties are placed in environment with natural contamination. Four coffee varieties are tested (BM71, BM139, POP 3303/21 and Jackson 2/1257). Plants were arranged in 3 randomized blocks. In each block, a variety is replicated 8 times. The experimental plot was located in Kigali city in Rwanda. Aphid populations were assessed at regular weekly intervals by counting living aphids and mummies. Results showed that the number of aphids vary with the blocks because of the soil fertility, the surrounding vegetation and the watershed effect. The variety BM139 seems to be the most attractive coffee variety while BM71 is less attractive to *T. aurantii*. Aphid population varied overtime. The considerable aphid populations occurred between 11th and 12th week, but decreased after 13th week probably due to predators and co-infection. Both environmental factors and coffee varieties influence attractiveness of *coffee arabica* to *T. aurantii*. This field experiment is being replicated in laboratory to confirm and precise our results.

Symbiont-conferred resistance compromises aphid control by parasitoids

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From a biocontrol perspective, the use of all-female parasitoids is desirable, because only females kill hosts. On the other hand, asexual parasitoids may fail to track evolutionary changes in the pest insect population. To compare biocontrol efficiency of asexual and sexual populations of the parasitoid wasp *Lysiphlebus fabarum*, population cages were set up with broad beans (*Vicia faba*) infested by large aphid populations (*Aphis fabae*) of high or low clonal diversity. Upon exposure to asexual or sexual parasitoids, population development was monitored during 13 weeks. Parasitoids went extinct in 12 out of 20 cages, and only 5 of the remaining 8 cages still contained significant numbers of parasitoids at the end of the experiment. Although there was some indication that asexual parasitoids might have a stronger effect on aphid population growth, biocontrol ultimately failed regardless of the parasitoid's reproductive mode. The reason was rapid selection for resistant aphids infected with the secondary bacterial endosymbiont *Hamiltonella defensa*. Thus, we had to reject our hypothesis that sexual parasitoids might be superior biocontrol agents when pest populations change, because even the sexual parasitoids did not adapt fast enough to the rapid evolution of symbiont-conferred resistance in the aphids. Given that a substantial proportion of pest aphids in the field harbour *H. defensa*, this work points to the need for careful exploration of predispositions towards resistance against parasitoids in integrated pest management.

Experimental evidence for the population-dynamic mechanisms underlying extinction cascades of carnivores

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Species extinction rates due to human activities are very high and initial extinctions can trigger cascades of secondary extinctions, leading to further erosion of biodiversity. A potential major mechanism for this is provided by the long-standing theory that the diversity of consumer species is maintained due to the positive indirect effects these species have on each other by reducing competition among their respective resource species. This means that the loss of one carnivore species could lead to competitive exclusion at the prey trophic level, leading to extinctions of further carnivore species. Evidence for these effects is difficult to obtain due to many confounding factors in natural systems but extinction cascades that could be due to this mechanism have been demonstrated in simplified laboratory microcosms. We established complex insect food webs in replicated field mesocosms and found that the over-harvesting of one parasitoid wasp species caused increased extinction rates of other parasitoid species, compared to controls, but only when we manipulated the spatial distribution of herbivore species such that the potential for interspecific competition at this level was high. This provides clear evidence for horizontal extinction cascades at high trophic levels due to the proposed mechanism. Our results demonstrate that the loss of carnivores can have widespread effects on other species at the same trophic level due to indirect population dynamic effects that are rarely considered in this context.

Species diversity of aphids and their natural enemies in crop associations

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Crop associations can be efficient to reduce aphid populations, by disrupting the visual and olfactory location of host plants. However, increasing the chemical and structural complexity of vegetation can also decrease the searching efficiency of predators and parasitoids, which are not always more abundant in complex habitats. Using attractive semiochemicals such as methyl salicylate (MeSA) combined with a crop association seems promising to maximise aphid control. We compared the abundances of aphids and their natural enemies between a wheat-pea mixture (with and without MeSA) and pure stands, using yellow traps and observations on plants. Significantly higher numbers of pea aphids (*Acyrthosiphon pisum*) were observed in the pure stand of pea compared with the mixture (with and without MeSA) in 2013 and 2014. Concerning aphid natural enemies, hoverfly larvae abundance was not significantly different between treatments in both years. Parasitoid mummies were significantly more abundant in the pure stand of pea compared with the mixture (with and without MeSA) in 2014. Fifty-two aphid species were collected in yellow traps, as well as 12 Aphidiinae and four *Aphelinus* species. *Acyrthosiphon primulae*, *Aphelinus daucicola*, *Aphelinus fusciscapus*, *Aphidius asteris*, *Aphidius eadyi*, *Metopolophium frisicum*, *Praon barbatum*, and *Trioxys auctus* were recorded for the first time in Belgium. Besides determining the effects of a wheat-pea mixture (with or without MeSA) on aphid control, this study contributed to increase the knowledge on aphid and their parasitoid diversity in Belgium, by recording new species and establishing the first Aphidiinae and *Aphelinus* national checklist.

Defensive symbiosis in aphids – linking within host mechanisms with ecology and evolution

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Since they appeared, no macro-organisms have been living free of bacteria. Adaptations to these hosted bacteria are thus probably as old as the origin of multicellularity. In particular, I will focus on physiological adaptations that might allow the host to control the evolution its mutualistic endosymbiotic bacteria when they are vertically transmitted from parent to offspring.

In general, mutualism is expected to be maintained by selection acting on hosts differing in their bacterial symbionts which affect their fitness. However, endosymbiotic bacteria undergo many cycles of reproduction during the host's life. If there is some diversity within the host – e.g. because of mutation or horizontal transmission – then particular bacteria could be selected during the host life. While selection acting at each host generation selects for bacteria improving the host's fitness, selection acting within the host favours bacteria which grow the fastest. This faster growth rate can be achieved by increased consumption of host resources, which might ultimately lead to the development of a selfish bacterial strain.

This problem can be compared to the development of "selfish cell lineages" such as cancer cells. Starting from what is known about protection against selfish cells, I will present mechanisms that could allow the host to prevent or control within-host evolution of symbionts to prevent evolution of selfishness.

Identification of membrane proteins from the aphid *Myzus persicae*’s gut implicated in polerovirus transmission

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Plant viruses are responsible for important worldwide economic losses. Since most phytoviruses are transmitted from plant to plant by vectors, chemical treatments are applied to reduce vector populations and to limit virus impact. Nevertheless, these methods are damageable for the environment and human health, and it is crucial to elaborate alternative strategies to reduce virus transmission. In our laboratory, we study poleroviruses, single stranded RNA viruses transmitted by the aphid *Myzus persicae* in a persistent and circulative manner. Virus particles, acquired by aphids on infected plants, cross intestinal cells by transcytosis to be released in the hemolymph. From there, they reach the accessory salivary gland cells and are transported through the cell by a similar mechanism. Particles are then released, together with saliva, into a plant during aphid feeding. Virus transport through the epithelia relies on the presence of specific virus receptors that are still unknown. In order to identify these receptors, virus partners identification was previously conducted by screening an aphid cDNA library with virus structural proteins using the yeast two hybrid system and three candidate proteins were identified. To increase the number of candidates, I performed in silico analyses of RNAseq data and identified a list of XX membranous intestinal proteins that may have receptor activity. We are now developing RNAi-based strategies to inhibit expression of the candidate genes in the aphid and evaluate their implication in virus transmission. The functional validation is based on the oral acquisition by aphids of double stranded RNA synthesized in plant or *in vitro*. This project could lead to the identification of aphid proteins involved in virus transmission but also proteins that ensure a vital function in the aphids. Inhibiting the expression of these proteins may ultimately result in the development of transgenic plants inhibiting virus transmission or displaying an insecticide effect.

Metagenomic analysis of the *Macrolophus pygmaeus* microbiome

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Plant bugs are heteropteran insects that belong to the large family Miridae. Most of the best known mirids are either harmful to agriculture (sap-sucking insects and vectors of plant diseases) or constitute auxiliary insects that prey on agricultural pests such as mites, psyllids, aphids and thrips. The present study describes the microbiome composition of the biological control auxiliary *Macrolophus* for the first time. The microbiome plays a crucial role in the growth, development and environmental adaptation of the insect host, and it is recognized as a major genetic resource of new molecules for the bio-processing industry. Here, we investigate the microbial diversity of this important group by using metagenome-sequencing on the HiSeq platform. The main bacterial taxa found include several species of Alphaproteobacteria belonging to the families Rickettsiaceae and Anaplasmataceae (with well---known genera such as *Wolbachia*) and Gammaproteobacteria belonging to the Enterobacteriaceae (a large family of Gram-negative bacteria that includes many symbionts). Our results expand recent studies based on PCR-cloning methods by the discovery of insect-bacteria associations previously unknown within the Miridae.

Exploring the place of the facultative symbiont *Serratia symbiotica* in ant–aphid mutualistic relationships

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Symbiotic associations with bacteria are ubiquitous in insects and are regarded as a source of evolutionary innovations that allowed these invertebrates to colonize a wide variety of environments. Thanks to their well-studied association with a wide range of symbiotic bacteria, aphids represent a valuable model to study symbiotic relationships in insects. *Serratia symbiotica* is one of the most frequent facultative symbiont found in aphids and includes endosymbiotic members as well as free-living strains. Despite the fact that biological roles of endosymbiotic *S. symbiotica* have been clarified (*e.g.* protection against heat shock and parasitoids), the role of the free-living strains remains unknown. *S. symbiotica* has already been detected in ants and recent studies have shown that the prevalence of this symbiont is much higher in aphid species that are tented by ants. Cases of bacteria having the ability to enhance the ant-aphid relationship by releasing semiochemicals have been described previously. Here we present a strategy to explore the potential role of *S. symbiotica* in ant–aphid mutualistic relationships.

Costs, benefits and immune defenses in the relationship between insects and symbiotic bacteria

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Symbiotic associations between microorganisms and insects are common in nature. These microorganisms can play important role of the evolution and ecology of their hosts by modifying their phenotype. Moreover, as these are heritable, they can be considered as a real adaptive potential.

In order to study how symbiotic microorganisms may persist and contribute to the evolutionary success of their host, aphids represent a valuable model thanks to their well-studied associations with a wide range of bacterial symbionts. Indeed, in addition to their obligate symbiont *Buchnera aphidicola* providing essential nutrients for their host survival, these pest insects may harbor various facultative symbionts conferring major phenotypic effects during environmental stresses.

Recently, several strains of the facultative symbiont *Serratia symbiotica*, have been isolated from *Aphis fabae*, making this symbiont one of the rare symbiont of insects that can be cultured in vitro and the only cultivable symbiont of aphids. These cultivable strains, which do not known their biological roles, are very interesting to study how could emerge a new symbiotic bacteria acquired from the environment and studying the defense mechanisms set up by the aphid to tolerate the presence of symbiotic bacteria in their tissues while protecting the host from pathogenic bacteria.

In this context, the aims of my Ph.D. are: **1)** to determine the biological role of cultivable *S. symbiotica* and its consequences on the host fitness, **2)** to understand the immune defense mechanisms set up at the hemolymph level when aphids have to face symbionts and pathogens, **3)** to understand the role of the oxidative environment in aphids for controlling invasive bacteria. This Ph.D. should clarify the role of *S. symbiotica* in aphids and the defense systems set up by these insects in the context of symbiotic interactions. Ultimately, it is about to understand how symbiotic associations have been able to keep up in the evolutionary history of insects.

A comprehensive proteome map of an insect symbiont (*Serratia symbiotica*) for better understanding the mechanisms of symbiosis

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Symbiotic bacteria are common in insects and can affect various aspects of their host biology. The effects of insect symbionts have been clarified for various insect symbiosis models, but there is still a limiting understanding of the molecular features that promote symbiosis. The recent finding of cultivable strains of *Serratia symbiotica* provides the opportunity to investigate the molecular features that determine the establishment and the persistence of symbionts in their host (i.e., symbiotic factors). In this work, a proteomic approach was used to establish a comprehensive proteome map of *S. symbiotica* CWBI-2.3T and to identify proteins having a relevant role in bacteria-host interactions. The majority of the 720 proteins identified was composed of housekeeping proteins or involved in the primary metabolism. Of these, dozens were identified as candidate proteins that might promote host colonization.

Optimization of FAIRE procedure to identify open chromatin regions in the pea aphid

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A vast diversity of living organism exhibits sexual dimorphisms between male and females individual. Sex determination involves different mechanisms depending on the species and may be based on the presence or absence of sex chromosomes. For instance, the sex of aphid individuals is determined genetically by the number of X chromosome copies, females being XX whereas males are X0. A recent report (Jaquiéry *et al.*, 2013) demonstrated that X chromosome global gene expression was significantly higher in males (haploid for the X) compared to females (diploid for the X). In other organisms such as *Drosophila melanogaster*, sex chromosomes are often characterized by specific regulatory mechanisms – including epigenetic regulations - to compensate for gene dose between the sexes.

We make the hypothesis that sex-specific expression patterns observed in *Acyrthosiphon pisum* might be explained by differential chromatin accessibility on the X chromosome. To test this hypothesis, we developed the Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE) methodology to isolate open chromatin regions from males and females. We then performed an Illumina high throughout sequencing on FAIRE DNA in order to study the X chromosome and Autosomes open chromatin profiles. Bioinformatics analysis and quantitative PCR validated this method. Further analyses of differential opening patterns between male and female individuals are ongoing.

Gall-inducing aphid depletes the reserves of its host tree

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Plant-manipulation by insects often creates nutrient sinks, which can affect the carbohydrates or nitrogen allocations within the host-plant. This can result in dramatic changes in the physiology, architecture and fitness of the host-plant. *Phloeomyzus passerinii* is considered a major pest of poplar since outbreaks of this aphid can lead to extensive tree mortalities. Within the bark of its host tree, it induces pseudogalls in the cortical parenchyma. In these pseudogalls the nutrient levels can be modified as amino acids accumulate, while starch granules tend to disappear. Since during outbreaks trunks can be covered with aphid colonies, we hypothesized that the dieback symptoms, and observed mortalities, could result from a significant alteration of nutrient allocations within the infested trees. We compared the non-structural carbohydrates content in the bark of uninfested and infested mature poplars. Samples were collected in autumn, during an outbreak, and in the following spring, when damage level on trees could be evaluated through the proportion of tree crowns that was still functional. The infestation dramatically reduced the starch content in autumn, and the reduction was correlated with the duration of infestation. It also reduced, but to a lesser extent, the content in soluble sugars. The following spring, the impact of infestation on carbohydrates content was still significant, and a strong correlation between starch content and damage levels was observed. This suggests that plant-manipulation by *P. passerinii* probably sharply modify the carbohydrates allocation, and depletes the reserves of its host tree, through direct or an indirect processes. In turn, the exhaustion of these reserves probably prevents bud breaking in the following spring, leading to tree death.

Hemipteran - host plant interactions: focus on some model insect saliva.

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Insect salivary components play important roles in plant-insect interactions, stimulating the plant defence or promoting infestation by manipulating plant physiology. Indeed, a variety of enzymes and organic components in saliva of herbivory insects can induce series of biochemical responses in damaged plants.

In our lab, several studies have been screening proteins in different aphid species saliva. The purpose of our current and future works is to widen that field of study to other piercing-sucking pests and host plants models.

Three models will be compared: the pea aphid (*Acyrtosiphon pisum* Harris), the invasive Asian brown marmorated stink bug (*Halyomorpha halys* Stål) and the invasive South American green vegetable stink bug (*Nezara viridula* L.).

A preliminary study showed differential expressions in bacterial proteins in salivary glands when *N. viridula* were exposed to tomato with or without glandular trichomes. This led to questioning whether the internal symbionts influence the adaptability of the pest to the host by manipulating saliva compounds. Then, a study can be led to assess the impact of symbiont profiles on pea aphid saliva proteins. We also began to analyse the impact of several diets on stink bugs salivary glands proteome.

We use especially proteomic techniques, such as SDS-PAGE and 2D-DIGE followed by LC-MS/MS and MALDI-TOF analysis, but also liquid chromatography gel free approaches. The perspective would be applying saliva or salivary glands extracts on plant in order to screen its defensive responses by complementary "omic" approaches and, in term, to identify candidate proteins playing a role in plant defence induction or bypass.

Plant-aphid-symbiont interactions: what are the factors maintaining their association?

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In this poster, we would like to give an overview of new research program on plant-aphid-symbiont interactions currently in progress at INRA Rennes. The pea aphid, *Acyrthosiphon pisum*, forms genetically distinct biotypes, and each biotype is specialized to one or a few legume species. *A.pisum* harbors obligate symbiont *Buchnera aphidicola* and often harbors secondary symbionts such as *Regiella*, *Hamiltonella* and *Serratia*. These secondary symbionts are not essential for aphid survival, but some of them are known to change aphid phenotype. Interestingly, each aphid biotype is associated with specific composition of the secondary symbionts. To examine involvement of secondary symbionts in plant-aphid interactions, we manipulated composition of secondary symbionts and examined impact of the symbionts on aphid fecundity. At the moment, we have not identified the condition in which a secondary symbiont plays a major role in aphid specialization to host plant. Therefore, we started to examine other factors (e.g. horizontal transfer of symbionts through plants) that might contribute to maintain the association between the aphid biotypes and the secondary symbionts. In the meantime, we examine aphid factors that are involved in the specialization to their host plants. As aphid saliva is direct interaction point between the aphids and the plant cells, we focus on the proteins that are secreted into saliva. By using genome resequencing data of various *A.pisum* lines, we identified the aphid salivary genes with biotype specific polymorphism. We are currently examining their functions in specialized and non-specialized plant species.

The influence of climate on autumn UK abundance of the *Rhopalosiphum padi.*

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Rhopalosiphum padi (L.) is a major cereal pest of temperate areas and an important vector of barley yellow dwarf virus (BYDV). Knowing the abundance of the cereal-colonising forms in autumn is of particular importance for decisions on BYDV control. In this study, we focus on evaluating the impact of previous winter temperature (mean daily temperature from December to February) and summer rainfall (mean daily rainfall from June to August) on the abundance of the female *R.padi* from September to November in the UK (log_{10} transformed). Aphid data came from the nine suction traps of the Rothamsted Insect survey, each of which represents 30 to 40 years' data. In order to compare regional differences, the whole dataset were divided into three groups, representing North, Middle, and South regions in the UK. Linear mixed effects models were carried out for each group, with suction trap locations as random effects, interactions among weather and year as fixed effects. Results show that previous winter temperature has strong negative relationships with *R.padi* autumn abundance for all regions. Summer rainfall has strong positive relationships with aphid abundance for the Middle and South regions, but weak relationships for the North. Interactions between temperature and year have positive relationships for all regions, and year effect itself has negative interactions with aphid abundance.

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