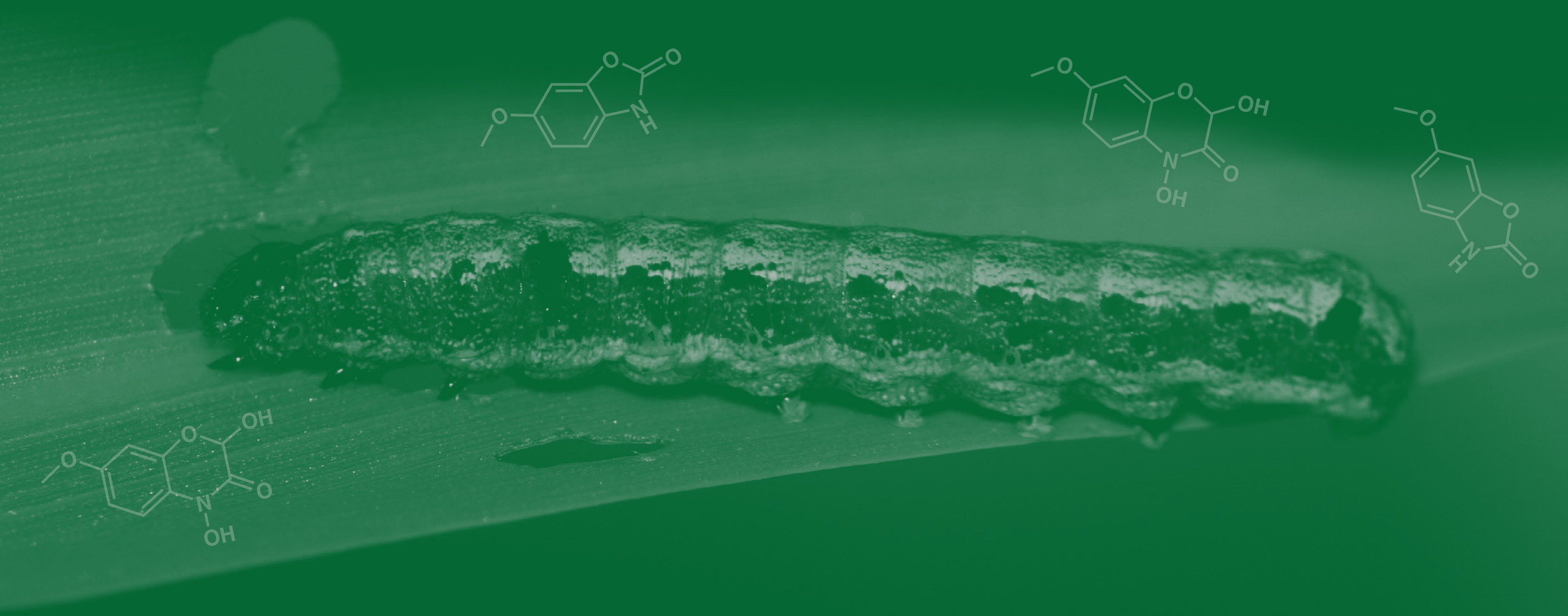
C3E NEWSLETTER 1/2015



CENTRE OF COMPETENCE IN CHEMICAL ECOLOGY

UNIVERSITÉ DE NEUCHÂTEL

FACULTÉ DES SCIENCES

TABLE OF CONTENTS 2

Cover illustration

Nest with begging chicks of the carrion crow *Corvus corone corone* alongside a chick of the great spotted cuckoo *Clamator glandarius*. The linked studies are presented on page 6. Photo credit Vittorio Baglione.

IMPRESSUM

Centre of Competence in Chemical Ecology (C3E)
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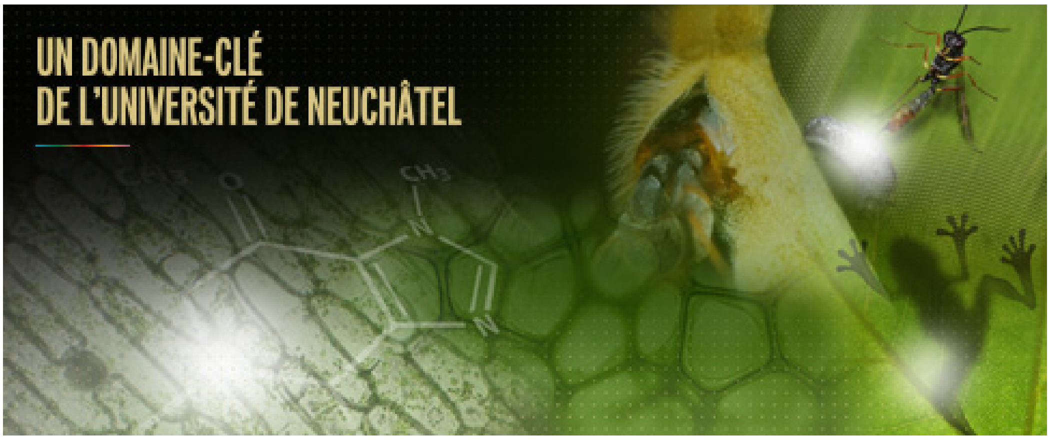

The new Centre of Competence in Chemical Ecology: from NCCR to C3E


Ted Turlings


Reinhard Neier


Felix Kessler

It is a great pleasure to write this editorial for the first newsletter of the Center of Competence in Chemical Ecology (C3E) of the University of Neuchâtel. C3E was created as a follow-up of the National Center of Competence in Research *Plant Survival*, in which research in chemical ecology played a dominant role. Chemical ecology is a highly interdisciplinary field that concerns chemically mediated interactions. It involves, for instance, chemists that identify and synthesize bioactive substances, physiologists that study the production and perception of these chemicals in plants and animals, but also ecologists that may study the impact of the chemically mediated interactions at individual or ecosystem level. Chemical ecology is also all-inclusive at the organismal level and relates to all taxa, ranging from bacteria to humans, and therefore is of great importance to all of biology. Most importantly, the research has great potential for application in the context of food security, ecosystem management, as well as in human and animal healthcare.

We are delighted that the University of Neuchâtel has recognized the importance of chemical ecology. We can claim to be the first and only university in Switzerland to include Chemical Ecology in its teaching curriculum and to specifically promote research in this field.

Together with participating partners at other Swiss universities we strive to follow in the footsteps of sister organizations worldwide, in order to conduct research and provide education in chemical ecology at the highest possible level. With several of these organizations we already have highly productive collaborative interactions. This is in particular the case for the Max Planck Institute for Chemical Ecology (Jena, Germany), Rothamsted Research (Harpenden, UK), the Insect Chemical Ecology, Ethology and Evolution group (Alnarp, Sweden), and Penn State University's Center for Chemical Ecology (University Park, Pennsylvania, US).

We are particularly proud of our association with the Neuchâtel Platform for Analytical Chemistry (NPAC), which provides analytical services to research institutions and industries worldwide. C3E and NPAC were created simultaneously. The highlights presented in the current newsletter attest to the diversity of successful activities to which we have so far contributed.

STEERING COMMITTEE

Ted Turlings (director C3E) *fundamental and applied research in chemical ecology*
Felix Kessler (director NPAC) *plant physiology*
Reinhard Neier *organic chemistry*

COORDINATION

Sonia Bolea (all centres of competence of the Faculty of Science of UniNE, until 2014)
Thomas Degen (C3E)
Teresa Fagundes (all centres of competence of the Faculty of Science of UniNE, from 2015)
Angela Köhler (C3E)

NPAC

Diego Carnevale *nuclear magnetic resonance unit* (from February 2015)
Claudio Dalvit *nuclear magnetic resonance unit* (until February 2015)
Gaétan Glauser *mass spectrometry and metabolomics unit*
Bruno Therrien *crystallography unit*
Armelle Vallat *mass spectrometry and metabolomics unit*

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Redouan Bshary *behavioural ecology*
Patrick Guerin *animal physiology*
Fabrice Helfenstein *evolutionary ecophysiology*
Pilar Junier *microbiology*
Brigitte Mauch-Mani *molecular and cell biology*
Sergio Rasmann *functional ecology*

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Urs Schaffner *CABI, Delemont, Switzerland*
Florian Schiestl *University of Zurich*
Jean-Luc Wolfender *University of Geneva*

Insects fight back: detoxification of maize defense metabolites increases performance of adapted herbivores

Angela Köhler

Daniel Maag

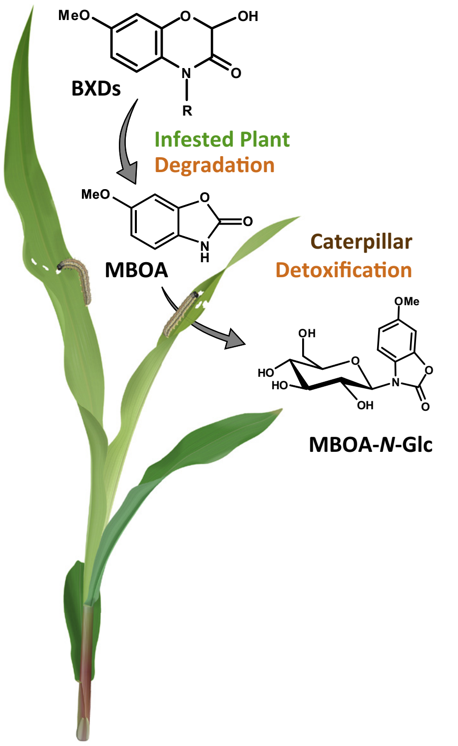
Spodoptera frugiperda

Spodoptera littoralis

Plants produce a variety of toxic or repulsive compounds to defend themselves against insect attacks. However, specialized insects have developed counter-strategies to cope with such chemical defenses, allowing them to become major agricultural pests. Maize plants produce benzoxazinoids (BXDs), a specific class of nitrogen-containing secondary metabolites that are stored as weakly active glucosides in the plant. Tissue damage initiates the conversion to active aglycones, which strongly affect the metabolism, behavior and fitness of the insect herbivores. The fall armyworm *Spodoptera frugiperda* has evolved mechanisms to circumvent negative effects of BXDs and causes considerable damage to maize crops in Central and North America.

Researchers in the FARCE laboratory study the role of maize BXDs at the plant-insect interface in an SNSF-funded Sinergia project headed by UniNE. They recently identified a novel detoxification product, MBOA-*N*-glucoside, in the feces of *Spodoptera frugiperda* caterpillars. *In vitro* assays showed that this molecule is formed enzymatically in the gut using the BXD breakdown product MBOA as precursor. Leaf-feeding *Spodoptera* larvae have a high detoxification capacity and tolerate MBOA in their diet, while larvae of the stem-feeding European corn borer *Ostrinia nubilalis* were hardly able to glucosylate MBOA and their growth was reduced. This may explain their different feeding behaviors in the field: *Spodoptera* larvae can defoliate well-defended maize seedlings, while *O. nubilalis* drills into the stem of older plants where BXD levels are much lower.

Another experiment revealed how the distribution of BXDs within young maize plants affects the foraging behavior of leaf-chewing caterpillars. Upon herbivore attack, young leaves increase the production of BXDs more strongly than older leaves. Bioassays showed that *S. frugiperda* larvae prefer and grow better on young leaves, while larvae of the related generalist species *Spodoptera littoralis* feed on older, less inducible leaves. Adaptation to BXDs thus allows *S. frugiperda* to feed in the protected plant whorl, causing the loss of valuable young leaf tissue. This preference was attenuated on BXD-free mutants, suggesting that BXDs may guide the adapted herbivore to nutritious young leaves. Understanding the precise role of BXDs in plant-insect interactions opens up interesting possibilities for new pest management strategies.



Maag D, **Dalvit C**, **Thevenet D**, **Köhler A**, Wouters FC, Vassão DG, Gershenzon J, Wolfender J-L, **Turlings TCJ**, Erb M, **Glauser G** 2014. 3-β-D-glucopyranosyl-6-methoxy-2-benzoxazolinone (MBOA-N-Glc) is an insect detoxification product of maize 1,4-benzoxazin-3-ones. ***Phytochemistry*** 102 : 97–105.

Köhler A, **Maag D**, **Veyrat N**, **Glauser G**, Wolfender J-L, **Turlings TCJ**, Erb M 2014. Within-plant distribution of 1,4-benzoxazin-3-ones contributes to herbivore niche differentiation in maize. ***Plant, Cell and Environment***: DOI 10.1111/pce.12464

The benefits of being cuckolded

Gregory Röder

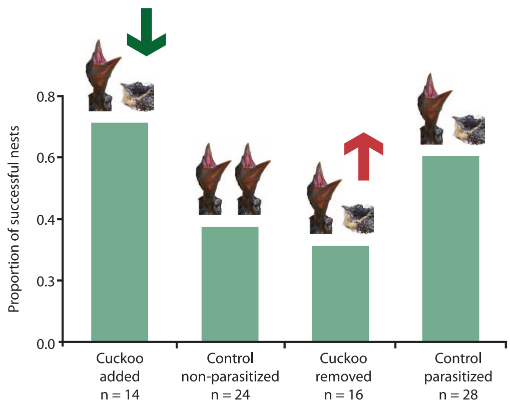
Clamator glandarius

Corvus corone corone

Initiated after a seminar given at the Institute of Biology by Vittorio Baglione in Fall 2010, a collaboration between the FARCE laboratory and researchers at two Spanish Universities came to fruition with articles published in *Science* and *Journal of Chemical Ecology*, respectively. The reported data suggest that raising a cuckoo chick does not necessarily have detrimental effects on the fitness of the foster parents.

Avian brood parasites lay eggs in the nests of other birds, which raise the unrelated chicks and typically suffer partial or complete loss of their own brood. The cuckoo family comprises a whole range of breeding habits: many species build their own nest, while a large minority engage in facultative or obligate brood parasitism. Contrary to the European common cuckoo, the great spotted cuckoo *Clamator glandarius* is a nonevicting brood parasite specialized on corvids, mainly magpies and carrion crows *Corvus corone corone.* Cuckoos strongly reduce magpie reproductive success, but apparently this does not occur in crows, whose larger offspring are often raised alongside the parasite.

The study carried out in Northern Spain investigated whether the great spotted cuckoo provides a benefit to crows and whether such benefit could derive from the ability of cuckoo chicks to deter predators with a noxious secretion that they release when harassed. Data from nest monitoring collected over 16 years were used to analyse the effect of the parasite on crow reproductive success. Cuckoo hatchlings were transferred into synchronous nonparasitized nests, whereas unmanipulated parasitized and nonparasitized nests served as controls. Among parasitized nests, those from which cuckoos were removed failed significantly more often than control, whereas among nonparasitized nests, the addition of cuckoo chicks resulted in significantly increased success. However, among all the nests that are successful, those with cuckoo chicks fledge fewer crows. The outcome of these counterbalancing effects fluctuates between parasitism and mutualism each season, depending on the intensity of predation pressure.



Probability of success of experimental and control nests. n = number of nests.

Using gas chromatography and mass spectrometry, Gregory Röder from the FARCE laboratory conducted the chemical analysis of the malodorous cloacal secretion, which revealed a mix of caustic and repulsive compounds, dominated by acids, indoles, phenols, and several sulfur containing compounds that are known to repel mammals and birds. Tests with treated chicken meat on species belonging to the main groups of crow nest predators (e.g. cats, birds of prey) provided evidence that the cuckoo secretion as well as synthetic mixtures of dominating volatile components thereof are highly repellent to those model predator species.

Canestrari D, Bolopo D, **Turlings TCJ**, **Röder G**, Marcos JM, Baglione V 2014. From parasitism to mutualism: unexpected interactions between a cuckoo and its host. ***Science*** 343: 1350-1352.

Röder G, Canestrari D, Bolopo D, Marcos JM, **Villard N**, Baglione V, **Turlings TCJ** 2014. Chicks of the great spotted cuckoo may turn brood parasitism into mutualism by producing a foul-smelling secretion that repels predators. ***Journal of Chemical Ecology*** 40: 320-324.

Plants getting ready for battle

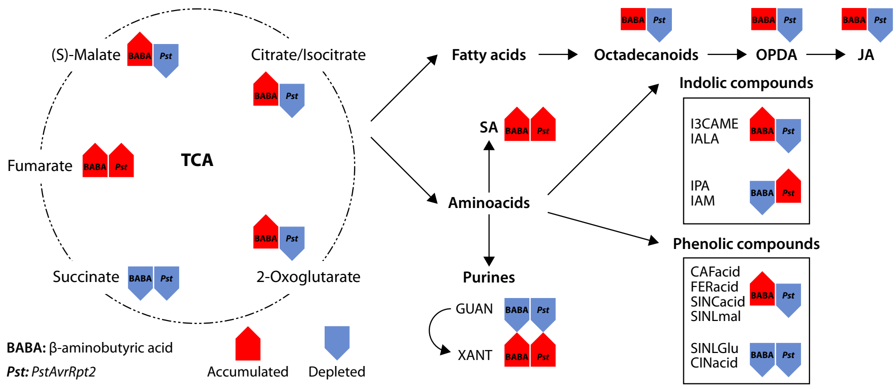
Victoria Pastor Fuentes

Andrea Balmer

Brigitte Mauch-Mani

As plants are generally immobile and cannot escape their attackers, they have evolved a variety of chemically mediated defense mechanisms to protect themselves against biotic and abiotic stresses. The first crucial step for a plant is to recognize that it is attacked. The speed by which the plant senses and accurately recognizes a specific stress or assesses how appropriate and successful its reaction will be determines its chances for survival. Failure to mount a timely or fitting response will, in case of pathogen attack, lead to colonization of the host tissues and hence to disease. The basal immunity of a plant contributes to slowing down the colonization process, but is generally too weak to effectively prevent disease. The level of basal immunity of a plant, however, can be enhanced through application of appropriate stimuli. This is commonly referred to as induced resistance. Plants have acquired the ability to widely improve their defensive capacity against a broad range of pathogens including viruses, fungi, oomycetes, and bacteria toward which they are genetically speaking susceptible. This defense has to be triggered by an inducing treatment such as inoculation with pathogens, rhizobacteria or a treatment with defined chemicals. During the initial phase of resistance induction, when the plant is preparing for a future attack, but has not yet been challenged by a pathogen is called the priming phase. This phase has had for a long time the status of a "black box", in which events important for the later defense reactions of the plant were taking place, but their exact nature remained unclear.

Highly improved and sensitive chromatographic techniques make it now possible to identify metabolites that accumulate during the priming phase. Our study has established a metabolomic profiling of the priming phase of *Arabidopsis thaliana* following induction of priming by two different priming cues, i.e., priming by inoculation with avirulent bacteria and chemical priming by soil-drench with BABA. The generated metabolic profile has revealed that this hitherto "silent" priming stage is finally not so silent. Priming causes a boost of the primary metabolism of the plant comparable to a stand-by state, that allows the plant to react much faster in case of an attack and thus greatly improves its survival chances.



Principal pathways/compounds that show significant changes after priming with 2 inducers. In priming experiments the stress is usually applied after 48 hpt. Rows represent the general changes after this period of time. Red shows the accumulated compounds and blue rows show the depleted compounds/pathways. TCA compounds are the main source for the synthesis of other metabolites playing a major role in defense. These compounds belong to the octadecanoid pathway (fatty acids) and amino acids. Both pathways are divided in subsequent pathways and compounds that are important in defense signaling and decide the final output when the plant meets a stress.

Pastor V, **Balmer A**, Gamir J, Flors V, **Mauch-Mani B** 2014. Preparing to fight back: Generation and storage of priming compounds. ***Frontiers in Plant Science*** 5: 295.

Modulation of hormone levels during aggressive interactions in territorial damselfish


Albert Ros


Redouan Bshary

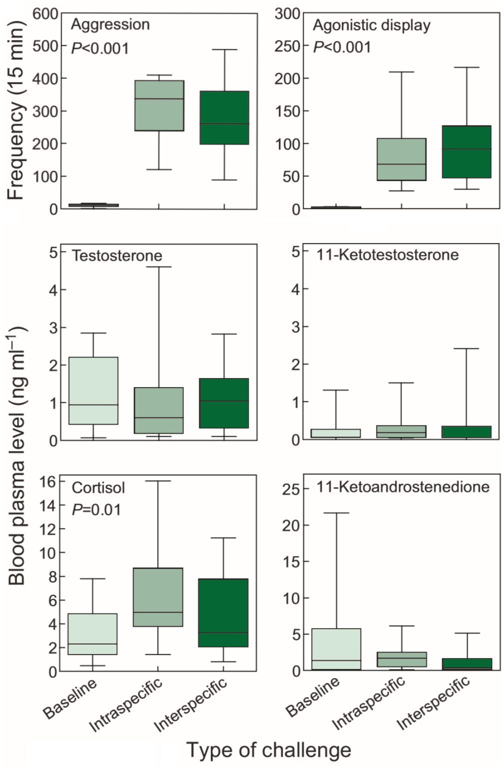

Stegastes nigricans


Abudefduf sexfasciatus

How individuals communicate and how information is transmitted are fascinating questions in biology. This involves mechanisms by which animals can send and detect signals and requires (social) learning. Interestingly individuals differ in how vigilant they are, and in the manner and vigour by which they produce their own signals. In the regulation and thus the individual variation in communication, steroid and peptidergic hormones have been shown to play a pivotal role. In our research, we focus on this hormonal regulation as a source of information that can help understand individual differences in behavioural dispositions, as in when individuals choose to cooperate, cheat or punish a partner/opponent. The hormonal analysis also answers questions on motivational differences between individuals in their aggressive attitudes to individuals of the same sex, of the different sex or even to individuals of a different species. Both experimental and hormonal factors are thought to synergise in order to coordinate the individual's functional social responses.

In collaboration with NPAC, the behavioural ecology group has developed tools and protocols to investigate the hormonal mechanisms that modulate animal behaviour and communication. This fruitful collaboration has spawned an increasing number of publications owing to the possibilities to measure steroid hormones from blood plasma, urine and faecal samples. The specificity and highly sensitive hormonal analysis of HPLC tandem mass spectrometry has enabled us to measure several steroid hormones from small quantities of blood. This is essential for sampling small animals and for minimizing stress when sampling. Sampling reef fish while scuba diving, for instance avoids the effects of stress due to transport or air exposure.

As an example of multihormone sampling we found in the year-round territorial species, *Stegastes nigricans*, that only cortisol levels increased after a 15 min exposure to a tube with an intraspecific or an interspecific intruder. Recently we showed that in a seasonal territorial species, *Abudefduf sexfasciatus*, androgen levels responded to intra- and interspecific challenges.



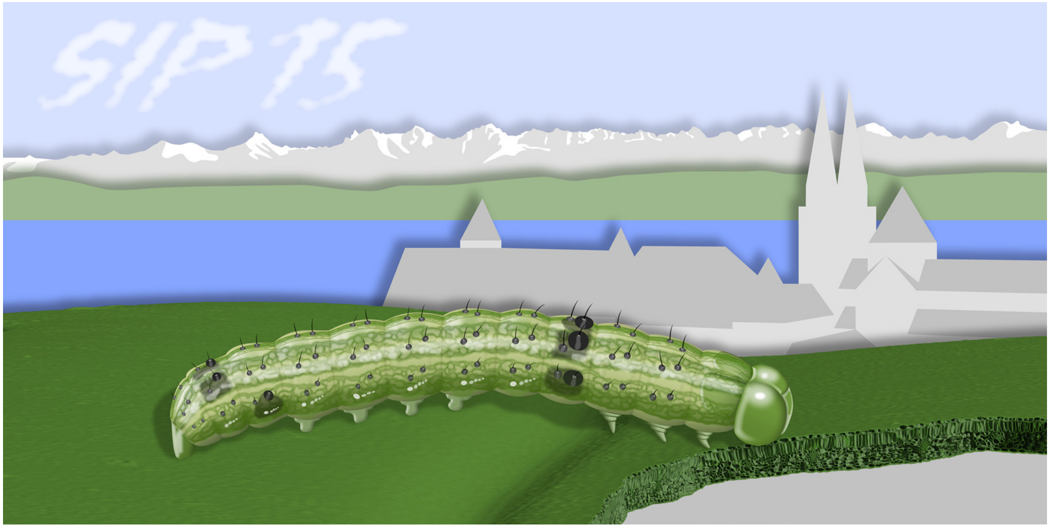
Territorial behaviors and steroid-hormone levels during baseline and simulated territorial intrusion tests in a guardener damselfish *Stegastes nigricans* in the Red Sea.

Ros AFH, Damjanovic K, **Glauser G**, **Bshary R** 2015. No scope for social modulation of steroid levels in a year-round territorial damselfish. ***Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*** 323, 80-88.

Ros AFH, Vullioud P, **Bruintjes R**, **Vallat A**, **Bshary R** 2014. Intra- and interspecific challenges modulate cortisol but not androgen levels in a year-round territorial damselfish. ***Journal of Experimental Biology*** 217, 1768-1774.

15th International Symposium on Insect-Plant Relationships

Betty Benrey



Organized by a committee consisting of Ted Turlings, Betty Benrey, Sandrine Gouinguené and Thomas Degen, the ***15th International Symposium on Insect-Plant Relationships*** SIP15 was held at the University of Neuchâtel on 17-22 August 2014, for the second time in Switzerland after the SIP9 in 1995. More than 200 researchers participated at the conference, and a total of 79 talks (15 by invited speakers) in 7 scientific sessions and 100 posters were presented.

Inauguration of the Park *Légende d'automne*

Pilar Junier

A delegation from the Laboratory of Microbiology at the inauguration of the Park *Légende d'Automne* on 19 September 2014. The bronze sculptures have benefited from a biopatina treatment, an innovative concept unique in Europe developed by the Laboratory of Microbiology. The sculpture park at promenade Schnetzler in Lausanne is accessible for blind and partially-sighted persons. An itinerary of 18 touchable scenes tells the *Légende d'Automne*, a tale written by artist and sculptor Sara H.

The Neuchâtel Platform of Analytical Chemistry (NPAC) – a state-of-the-art service and research facility

Neuchâtel Platform of Analytical Chemistry

The NPAC was created in 2013 by the fusion of the Service Analytique Facultaire (SAF) of the University of Neuchâtel and the Chemical Analytical Service (CAS) of the Swiss Plant Science Web. To further expand the platform and strengthen its position, substantial investments for equipment are planned for the midterm future (2017-2020) in the context of the Roadmap for Infrastructure (Swiss National Science Foundation and State Secretariat for Education, Research and Innovation). While NPAC is an entity separate from C3E in terms of organisation, there are very strong links between both units as far as research is concerned. The primary mission of the NPAC is to serve as a support for research projects for the local and external academic community on a collaborative basis. The scientists running the platform develop and optimise methods for specific applications, and provide advice and assistance for sample preparation, as well as interpretation of the obtained data. They perform more than 25'000 analyses per year and in 2014 contributed to 25 scientific publications. NPAC is currently organized in three units:


Armelle Vallat


Gaétan Glauser

Mass Spectrometry and Metabolomics Unit

Armelle Vallat and Gaétan Glauser are actively contributing to advances in applied mass spectrometry through the development of novel methods for specific biological and chemical issues. The detection, identification, and quantification of biologically active compounds from complex matrices are challenging and time-consuming tasks for life science researchers. Advances in this field have generally been related to advances in analytical chemistry techniques. One major recent development is the emergence of **metabolomics**, which can be considered as the comprehensive analysis of low molecular weight metabolites (<1500 Da) in given organisms in different physiological states. The capacity of mass spectrometry to detect multiple compounds with high sensitivity makes it a key tool in metabolomics.


Claudio Dalvit


Diego Carnevale

Nuclear Magnetic Resonance Unit

This unit has been managed by Claudio Dalvit, who has been succeeded by Diego Carnevale as of February 2015. Nuclear Magnetic Resonance (NMR) spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information of molecules. It is based on the physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms. The detection of NMR signals is achieved by polarizing the magnetic nuclear spins with a constant magnetic field $\mathbf{H}^0$ and by perturbing these nuclear spins with radio frequency (RF) pulses. The required perturbing frequency depends on the static magnetic field ($\mathbf{H}^0$) and the nuclei of observation (e.g. ^{1}H, ^{13}C, ^{19}F, ^{31}P). Detailed information on the topology, dynamics and three-dimensional structure of molecules in solution and the solid state can be obtained with NMR. The versatility of the technique allows its application in many different research areas both in academia and industry.


Bruno Therrien

Crystallography Unit

Bruno Therrien is in charge of the chrystallography Unit. X-ray crystallography is a tool used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal. From this electron density, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their disorder and various other information.

Services of the Neuchâtel Platform of Analytical Chemistry

Equipment

Waters
Acquity UPLC-Synapt G2 high resolution mass spectrometer

Nano-Acquity UPLC-Ion-Key-Xevo TQ-S triple quadrupole mass spectrometer (available 2015)

Semi-preparative HPLC-UV

AB Sciex
Ultimate® 3000 RSLC-4000 QTRAP mass spectrometer

Agilent
GC 7890B - MSD 5977 (available 2015)

HPLC-DAD 1100

Bruker
DPX 400 MHz equipped with a BBO probehead

Avance II 400MHz equipped with a BBOF+ probehead

Stoe Mark II-Image Plate Diffraction System, using Mo-Ka graphite monochromated radiation

for Academics / for Industry

Mass Spectrometry and Metabolomics Unit

For Academics: The Mass Spectrometry and Metabolomics Unit has strong expertise in the **targeted** and/or **untargeted** analysis of small molecules present in various biological matrices. UHPLC-DAD-MS/(MS) is used for the quantification of selected compounds with standard methods (e.g. phytohormones, prenyllipids, glucosinolates benzoxazinones, steroid hormones malondialdehyde, organic acids) or specifically developed non-routine methods (e.g. alkaloids, cyanogenic glycosides, flavonoids, napthoquinones). To provide the broadest picture possible of metabolomes in untargeted analysis, we use reverse phase ultra-high pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS).

For Industry: In recent years, we have established collaborations with about twenty companies, including pharmaceutical, chemical, biomedical, food, and nano-technology companies. In addition to the services for academics (see left), non-routine analyses in various application domains, e.g. phosphorus chemicals, polymers, trace elements, metabolism studies, may be carried out on our high-end instruments (LC-MS, GC-MS and ICP-OES). We also offer collaboration/assistance for solving your challenging issues.

Nuclear Magnetic Resonance Unit

For Academics: We have a long experience in the analysis of small molecules and their interactions with biomolecular receptors. NMR support includes:

- NMR structure elucidation of small molecules and natural products
- Conformational dynamics studies of small molecules
- 3D structure determination of peptides in solution
- NMR-based fragment screening
- Protein-ligand and protein-protein interaction studies
- NMR biochemical assays

For Industry: In addition to the services for academics (see left) we perform:

- Compound stability studies
- Compound quality control analysis
- Spectral interpretation

Crystallography Unit

For Academics: We possess a long experience in handling sensitive molecules, in mounting small crystals and in solving the molecular structures of organic, inorganic and organometallic compounds.

For Industry: We offer a complete service for structure solution and refinement, at room or low temperature.

Examples of the diversity of studies NPAC was involved in

Fabrice Helfenstein

Jenni-Eiermann S, **Helfenstein F**, **Vallat A**, **Glauser G**, Jenni L. 2015. Corticosterone: Effects on feather quality and deposition into feathers. ***Methods in Ecology and Evolution*** 6: 237–246.

Measuring the concentration of the glucocorticoid hormone corticosterone in feathers is a way to reveal past events of increased stress during feather growth in birds. Quantification with ultra-high performance liquid chromatography–tandem mass spectrometry was used to double check whether corticosterone is reliably measured with an enzyme immunoassay and with the analysis of injected tritium-labelled corticosterone.

Lucia Eugeni-Piller

Eugeni-Piller L, **Glauser G**, **Kessler F**, **Besagni C**. 2014. Role of plastoglobules in metabolite repair in the tocopherol redox cycle. ***Frontiers in Plant Science*** 5: 298. doi: 10.3389/fpls.2014.00298

Plants increase the production of membrane soluble antioxidants such as tocopherols to limit damage by excessive light. In this study, prenyllipids (α-tocopherols, α-tocopherol quinone, plastochromanol, plastoquinone) were quantified using reverse-phase ultra-high pressure liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPLC-QTOFMS). Absolute concentrations were calculated based on calibration curves obtained from pure standards.

Patrick Guerin

Kessler S, Gonzalez J, Vlimant M, **Glauser G**, **Guerin PM** 2014. Quinine and artesunate inhibit feeding in the African malaria mosquito *Anopheles gambia*: the role of gustatory organs within the mouthparts. ***Physiological Entomology*** 39: 172-182.

The study describes a membrane feeding assay to test the effects of the antimalarial drugs quinine and artesunate on mosquitos. Artesunate is not stable in blood and is converted into dihydroartemisinin. To evaluate the kinetics of this conversion in blood samples, both compounds were quantified by UHPLC-QTOFMS.

Sergio Rasmann

Megali L, **Glauser G**, **Rasmann S** 2014. Fertilization with beneficial microorganisms decreases tomato defenses against insect pests. ***Agronomy for Sustainable Development*** 34: 649-656.

The study demonstrated that the application of biofertilizer might produce unintended negative effects on a crop plant: tomato plants inoculated with beneficial microorganisms were impaired in the induction of the toxic glycoalkaloid molecule tomatine and the defense-related phytohormone jasmonic acid after herbivore attack. The content of defensive secondary metabolites in leaves and phytohormone accumulation was assessed using UHPLC-QTOFMS.

Lassaâd Belbahri

Daassi D, **Belbahri L**, **Vallat A**, Woodward S, Nasri M, Mechichi T 2014. Enhanced reduction of phenol content and toxicity in olive mill wastewaters by a newly isolated strain of *Coriolopsis gallica*. ***Environmental Science and Pollution Research*** 21:1746-1758.

The ability of new isolates of fungal strains to degrade olive mill waste water (OMW) and to withstand the toxic effects of the initially high phenolic content was studied. The strongest reduction in the concentrations of phenolic compounds was obtained with *Coriolopsis gallica* as shown by the analysis of extracts from treated and untreated OMW with reversed-phase HPLC-DAD.