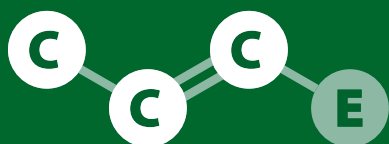


C<sub>3</sub>E

# NEWSLETTER 2021



CENTRE OF COMPETENCE IN CHEMICAL ECOLOGY



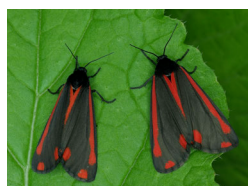
FACULTÉ DES SCIENCES

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**IMPRESSUM**

Centre of Competence in Chemical Ecology (C<sub>3</sub>E)  
 Faculty of Sciences, University of Neuchâtel  
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Cover image  
 Cinnabar moths *Tyria jacobaeae*  
 (see Page 11)



## Continuous output



Thomas Degen

At the time when we are writing this, Switzerland is in the midst of a strong fifth wave of COVID-19 infections, with the new variant omicron having just arrived. Almost two years after the outbreak of the global pandemic, the situation at the University had been slowly normalizing, with the majority of students back in the lecture rooms. Before, teaching was most strongly affected by the measures taken against the spread of the coronavirus, but research was also touched to some degree, notably when it came to planned field work abroad, that could not be carried out for some time. One activity that was hardly hampered, was the steady scientific output in terms of publications, as witnessed by the sample of articles that we have chosen to be featured in this newsletter, a selection that is admittedly somewhat biased towards those published in prestigious journals. The articles cover a wide spectrum of topics, which reflects the diversity of the research conducted by the groups participating in C<sub>3</sub>E. None of these papers have been authored exclusively by scientists at University of Neuchâtel, but all of them involve partners at other research institutions in Switzerland or abroad. This highlights once again the importance of collaboration, as we have already previously pointed out. The fact that this includes cooperation with Chinese researchers also goes to show that fruitful

exchange is possible on a personal level even in times of rising political conflict.

Another constant that we wish to keep on emphasizing is the crucial role that the Neuchâtel Platform for Analytical Chemistry (NPAC) plays for C<sub>3</sub>E and its members. The NPAC contributes the majority of chemical analytical data for papers that are published in the framework of C<sub>3</sub>E, such as those described on pages 5-8. In addition to providing chemical analytical data for research projects of UniNE and beyond, the NPAC is also actively engaged in the development of methods for the newest generation of instruments used in mass spectrometry-based metabolomics. Based on its experiences it has published a tutorial which provides guidelines for data-dependent acquisition (see page 12). The success of the NPAC can be attributed to the competence and efficiency of the scientists that run the platform, as well as to its state-of-the-art equipment and affordable pricing scheme.

As always, we close the newsletter with a presentation of new projects that have been awarded to researchers participating in C<sub>3</sub>E or launched by them during the year. We hope that you can find some inspiring information in this newsletter and that you can enjoy the forthcoming Christmas season despite the COVID-19 restrictions you may have to endure.

## STEERING COMMITTEE

**Ted Turlings** (director C<sub>3</sub>E) *fundamental and applied research in chemical ecology (FARCE)*  
**Felix Kessler** (vice-rector research UniNE) *plant physiology*  
**Stephan von Reuss** (director NPAC) *bioanalytical chemistry*

## COORDINATION

**Thomas Degen** *chemical ecology*

## NPAC

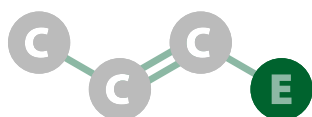
**Gaétan Glauser** *mass spectrometry and metabolomics unit*  
**Armelle Vallat** *mass spectrometry and metabolomics unit*  
**Sylvain Sutour** *nuclear magnetic resonance unit*

## PARTICIPANTS UNINE

**Betty Benrey** *evolutionary entomology*  
**Redouan Bshary** *behavioural ecology*  
**Daniel Croll** *evolutionary genetics*  
**Edith Joseph** *chemistry for cultural heritage*  
**Pilar Junier** *microbiology*  
**Jacob Koella** *ecology and epidemiology of parasites*  
**Ricardo Machado** *experimental biology*  
**Brigitte Mauch-Mani** *molecular and cell biology (retired in 2021)*  
**Sergio Rasmann** *functional ecology*  
**Gregory Röder** *chemical ecology*

## EXTERNAL PARTNERS

**Consuelo De Moraes & Mark Mescher** *ETH-Zürich*  
**Matthias Erb** *University of Bern*  
**Ted Farmer** *University of Lausanne*  
**Cris Kuhlemeier** *University of Bern*  
**Felix Mauch** *University of Fribourg*  
**Heinz Müller-Schärer** *University of Fribourg*  
**Philippe Reymond** *University of Lausanne*  
**Christelle Robert** *University of Bern*  
**Urs Schaffner** *CABI, Delémont, Switzerland*  
**Florian Schiestl** *University of Zurich*  
**Jean-Luc Wolfender** *University of Geneva*



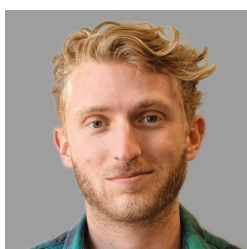
## A plant receptor for an insect elicitor



Adam Steinbrenner



Eric Schmelz



Julien Dongiovanni



Ted Turlings

Researchers from the FARCE laboratory and the NPAC made a modest contribution to a collaborative study conducted under the lead of researchers at the University of California San Diego, Eric Schmelz and Adam Steinbrenner, who was recently appointed assistant professor at the University of Washington, Seattle. They achieved a major breakthrough in the search for a plant receptor involved in the perception of herbivory, which was published in *PNAS*.

Plants respond to biotic attack using an immune system that involves receptors to recognize molecules characteristic for specific attackers, be it pathogens or herbivores. Immune recognition of pathogen-associated molecular patterns (PAMPs) is often mediated by pattern recognition receptors, but precisely defined receptors for herbivore-associated molecular patterns (HAMPs) had remained elusive. Eric Schmelz had already been the driving force behind the discovery of inceptins, peptid elicitors found in the oral secretions of Lepidopteran species, which are proteolytic fragments that are derived from chloroplastic ATP synthase of their host plants and trigger strong defence responses in legumes. Caterpillars feeding on cowpea *Vigna unguiculata* provoke the production of the dominant inceptin, 11-amino acid peptide termed Vu-In. The authors hypothesized that legumes encode an inceptin receptor, which enables the recognition of Vu-In. Using forward-genetic mapping of inceptin-induced plant responses, they succeeded in identifying a receptor-like protein termed INR from legumes in the subtribe Phaseolinae that confer inceptin-induced responses and enhanced defence against armyworms. INR is only present in the genomes of cowpea, common bean, and related legumes, but when the encoding genes were transferred to tobacco plants, *Spodoptera* caterpillars feeding on these transgenic plants showed as well



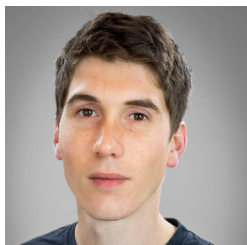
Caterpillar of the beet armyworm *Spodoptera exigua* feeding on a bean leaf.

reduced growth rates. Julien Dongiovanni, a Master's student from the Farce group, collected oral secretions from *Spodoptera* caterpillars that had consumed leaves of cowpea and of two species of tobacco, *Nicotiana tabacum* and *Nicotiana benthamiana*. Gaetan Glauser of the NPAC analysed the samples for the presence of inceptins. He could detect the variant Nb/Nt-In with a single amino-acid substitution in oral secretions of tobacco-fed armyworm larvae at similar levels to Vu-In in secretions from cowpea-fed caterpillars.

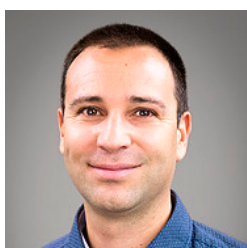
The reported findings represent a major advance in the understanding of mechanisms underpinning plant-herbivore recognition, and illustrate the complexity of chemical warfare between plants and insects. The discovered receptor mediates plant defence in response to a common oral secretion pattern in Lepidoptera, and the fact that it was functional when transferred into tobacco plants suggests that it may be used to engineer insect resistance in species within and across plant family boundaries.

Steinbrenner AD, Muñoz-Amatriaín M, Chaparro AF, Aguilar-Venegas JM, Lo S, Okuda S, Glauser G, Dongiovanni J, Shi D, Hall M, Crubaugh D, Holton N, Zipfel C, Abagyan R, Turlings TCJ, Close TJ, Huffaker A, Schmelz EA 2020. A receptor-like protein mediates plant immune responses to herbivore-associated molecular patterns. *PNAS* 117: 31510-31518. DOI:[10.1073/pnas.2018415117](https://doi.org/10.1073/pnas.2018415117)

## Assessing potential intricacies of climate change affecting an alpine ecosystem



Alan Kergunteuil



Sergio Rasmann

Members of Sergio Rasmann's Functional Ecology research group and of NPAC teamed up with colleagues from the ETH Zurich to study the potential effects of climate change on the compositions of alpine plant communities found growing in the Swiss Prealps. They experimentally simulated the anticipated faster upward range shift of animals than plants by translocating low-elevation herbivorous insects to alpine grasslands. They reported their important findings in an article published in *Science*.

At higher elevations, plants generally experience lower levels of herbivory allowing them to relax their chemical defences. Climate-driven elevational range shifts of herbivorous insects can occur much faster than those of plants. This generates opportunities for the insects to feed on poorly defended plants, which in turn could change the structure and functioning of alpine plant communities. The experiment consisted in moving grasshoppers from the collection site at an altitude of 1400 m into cages placed at three locations above the treeline, at 1800, 2070, and 2270 m in the Swiss Prealps. Over four growing seasons, the differential

effects of increased herbivore pressure and of warming, mimicked with open top chambers, on the experimental plant communities were assessed. Changes in biomass, structure, and composition of the plant communities were measured, considering numerous physical and chemical leaf traits such as toughness, content of silica, chlorophyll and secondary metabolites. The increased herbivory by the newly introduced grasshoppers disrupted the vertical organization of the plant canopy, much more than warming alone. Such an effect was mostly driven by the fact that the translocated orthopterans retained their dietary preferences and fed on high-elevation plants with functional traits matching their low-elevation host plants. This reduced the biomass of dominant alpine plant species and favoured the establishment of resistant small-stature plant species, thereby affecting plant species richness and community composition. The authors conclude that novel biotic interactions represent a neglected but major driver of ecosystem modifications under climate change, potentially outweighing the direct effect of temperature rise.



One of the three experimental sites located above the treeline in the western Swiss Alps. Photo by Patrice Descombes.

Descombes P, Pitteloud C, **Glauser G**, **Defosse E**, **Kergunteuil A**, Allard P-M, **Rasmann S**, Pellissier L 2020. Novel trophic interactions under climate change promote alpine plant coexistence. *Science* 370: 1469–1473. DOI: [10.1126/science.abd7015](https://doi.org/10.1126/science.abd7015)

## A wide-angle picture of phytochemical diversity and its determinants



Emmanuel Defosse

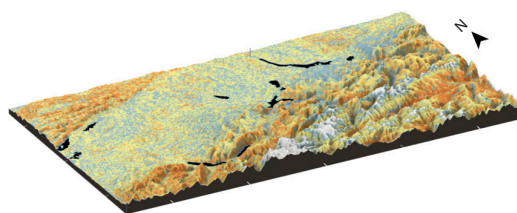
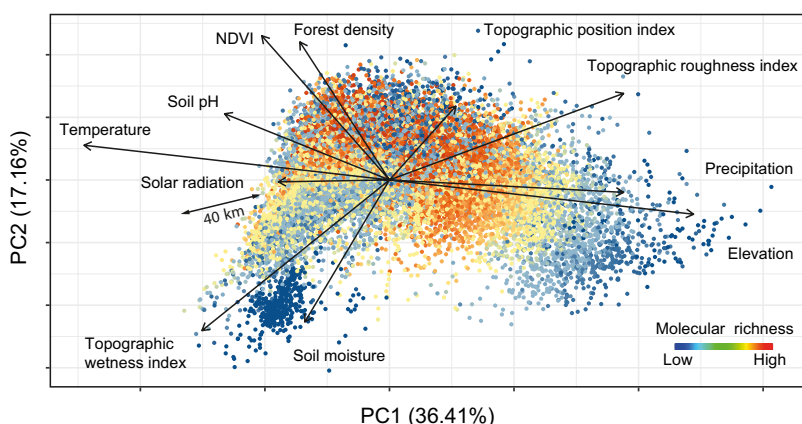


Pilar Fernandez-Conradi

Emmanuel Defosse, Pilar Fernandez-Conradi and Sergio Rasmann from the Functional Ecology Lab, in collaboration with Gaétan Glauser from NPAC, and with colleagues from the ETH Zurich and the University of Geneva, co-authored a paper in PNAS, which proposes an innovative holistic approach for predicting phytochemical diversity across the angiosperm phylogeny, and also to spatially map it at the landscape level. The evolutionary origin of this diversity and its variation across ecosystems is still not fully understood. The authors hypothesized that the amount of phytochemical diversity in a given habitat is not only related to the local plant species diversity, but is also constrained by other ecological factors, especially trophic, climatic, edaphic, and topographic variation.

The researchers aimed to address the problem in a comprehensive way: focussing on grassland vascular plants occurring from the foothills to the alpine vegetation zones, they screened the metabolomes of 416 species, thereby covering 55% of the genera recorded in this type of vegetation across the Alpine arc. Leaves were collected across six elevation gradients encompassing a range of soil conditions and four alpine bioclimatic regions. Leaf methanolic extracts were then analysed using UHPLC-quadrupole time-of-flight mass

spectrometry in untargeted fragmentation mode. The spectral dataset was organized through molecular networking and processed into a representation of phytochemical diversity for each species. These chemical analytical data were then combined with phylogenetic information, topographic, edaphic, and climatic variables, species distribution models and ensemble machine learning to extrapolate phytochemical diversity across the landscape. Spatial mapping of phytochemical diversity revealed that plant assemblages found at lower to middle elevations and with more alkaline soils possessed greater phytochemical diversity, whereas alpine habitats harboured higher phytochemical endemism. The sophisticated methodological framework developed in this study can thus be used to locate hotspots of phytochemical diversity in the landscape, independently of plant species taxonomic identity. Accordingly, such an approach offers promising perspectives in drug discovery programs, as well as for habitat conservation efforts worldwide. It represents an inspiring and welcome addition to the toolset of chemical ecologists for future analyses. In a [tweet](#) on 4.3.2021, Richard Grenyer from the University of Oxford has single-handedly declared the article “paper of the year so far”! We do not know whether it has been superseded in the meantime...



**Geographic mapping of phytochemical diversity.** Left: PCA of phytochemical diversity data extrapolated from a random sample of 20,000 geographic pixels (100-m resolution) from the map to the right. Arrows represent loadings for various environmental variables; Right: Phytochemical diversity across 20,000 km<sup>2</sup> in Switzerland.

**Defosse E**, Pitteloud C, Descombes P, **Glauser G**, Allard P-M, Walker TWN, **Fernandez-Conradi P**, Wolfender J-L, Pellissier L, **Rasmann S** 2021. Spatial and evolutionary predictability of phytochemical diversity. *PNAS* 118: e2013344118. DOI: [10.1073/pnas.2013344118](https://doi.org/10.1073/pnas.2013344118)

## Coming out of the dark, going green



Rosa Pipitone

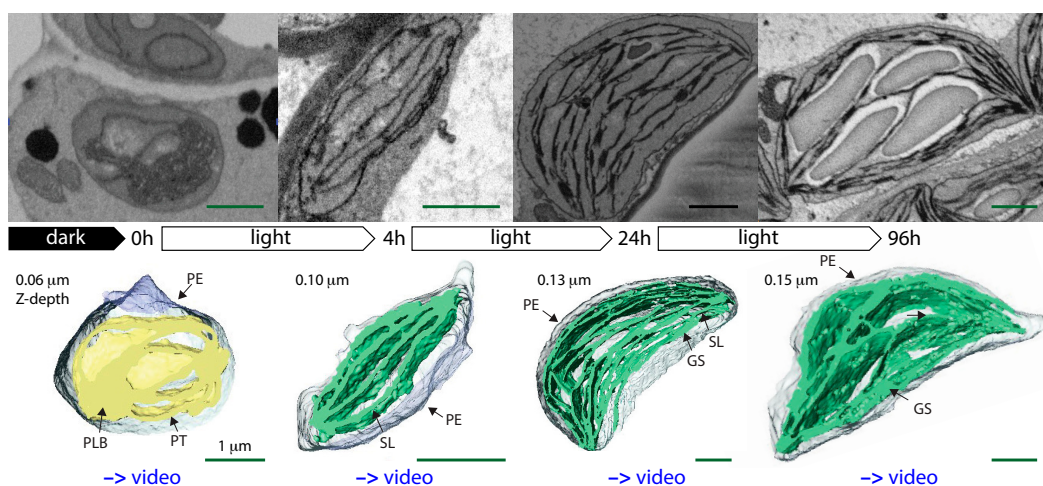


Felix Kessler

Rosa Pipitone has published ground-breaking findings from her thesis, carried out under the co-supervision of Emilie Demarsy, a former SNF Marie Heim-Vögtlin scholar at UniNE and now lecturer in Geneva, and Felix Kessler, head of the plant physiology lab. The study also involved cooperation with researchers at the NPAC, the ETH Zurich and the University of Grenoble, France. Combining different methodological approaches, the scientists describe in unprecedented detail a process in plant cells that is of utmost developmental and ecological importance: the transformation of etioplasts (precursors of chloroplasts in the dark) into photosynthetically active chloroplasts, when after germination a plant grows out of the soil and reaches the daylight.

Working with *Arabidopsis thaliana* seedlings, the scientists strived to capture a comprehensive picture of the light-triggered chloroplast differentiation in space and time. Using Serial Block Face Scanning Electron Microscopy (SBF-SEM), which allows collecting a

tomographic sequence of hundreds of images of the same area, they generated a series of 3D reconstructions of chloroplasts embedded in the plant cells. This made it possible to assess chloroplast number and volume and the extent of envelope and thylakoid membrane surfaces. These ultrastructural data were complemented with whole proteome and quantitative lipid data. The NPAC analysed the abundance and kinetics of accumulation of galactolipids, which constitute the bulk of the thylakoid membranes, and could show that galactolipid composition differs between etioplasts and chloroplasts. Based on the integration of all the acquired information, the authors could identify two distinct phases of chloroplast biogenesis: an initial photosynthesis-enabling “Structure Establishment Phase” followed by a “Chloroplast Proliferation Phase” during cell expansion. They also appreciated the astonishing speed at which the observed transformation takes place: the etioplast needs less than 24h to turn into a fully functional chloroplast.



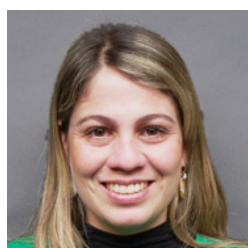
**3D reconstructions of chloroplast thylakoid networks during de-etiolation.** Top: Scanning electron microscopy images of representative etioplasts and chloroplasts from 3-day-old, dark-grown *Arabidopsis thaliana* seedlings illuminated for variable times (0-96h) in continuous white light. Bottom: Partial 3D reconstruction of thylakoid membranes (green) at different Z-depth (0.06 -0.15 μm). PLB: prolamellar body; PT: prothylakoid; PE: plastid envelope; SG: starch grain; GS: grana stack; SL: single lamella. Consult videos to get a more complete picture.

**Pipitone R, Eicke S, Pfister B, Glauser G, Falconet D, Uwizeye C, Pralon T, Zeeman SC, Kessler F, Demarsy E** 2021. A multifaceted analysis reveals two distinct phases of chloroplast biogenesis during de-etiolation in *Arabidopsis*. *eLife* 10: e62709. DOI: [10.7554/eLife.62709](https://doi.org/10.7554/eLife.62709)

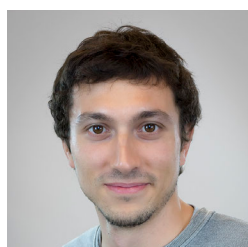


CO<sub>2</sub> as a cue

Ricardo Machado



Carla Arce

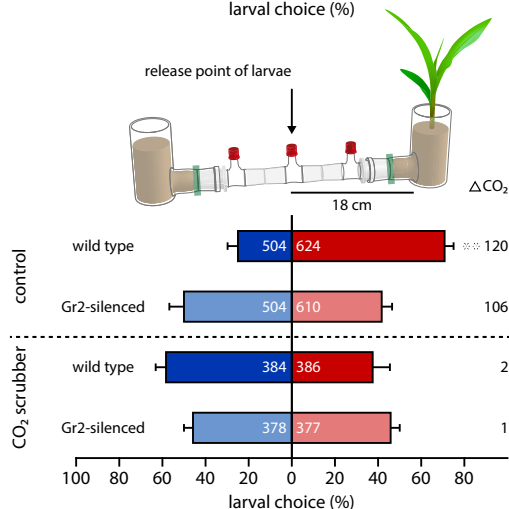
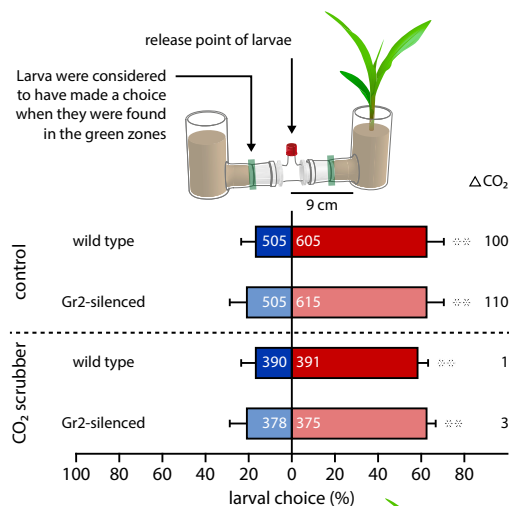


Geoffrey Jaffuel

Carbon dioxide is more than a greenhouse gas contributing to climate change, a nutrient for plants and a waste product of respiration, it can also act as a cue that plays a role in insect-plant relationships. This phenomenon was the subject of a study led by Ricardo Machado from the Experimental Biology lab in collaboration with Carla Arce and Geoffrey Jaffuel from the FARCE lab and with members of the Biotic Interactions lab of the University of Bern. They examined how CO<sub>2</sub> perception affects the interaction of the western corn rootworm (WCR) *Diabrotica virgifera virgifera* with the roots of its host plant. The results of this investigation were reported in an article published in *eLife*.

WCR larvae feed almost exclusively on maize roots in agricultural settings and can cause major yield losses. They rely on volatile organic compounds to locate host plants. To understand the relative importance of CO<sub>2</sub> along with these attractive volatiles for WCR foraging in the soil, the researchers reduced the expression levels of three putative WCR CO<sub>2</sub>-receptor-encoding genes through RNA interference. In that way they succeeded in identifying DvGr2 as a gene essential for CO<sub>2</sub> perception. Knocking down DvGr2 expression did not alter the capacity of WCR larvae to respond to important chemical cues other than CO<sub>2</sub>. In an olfactometer, DvGr2-silenced larvae did not respond to enrichment with synthetic CO<sub>2</sub> at any of the tested concentrations, whereas wild type larvae showed characteristic dose-dependent behavioural responses, being attracted to CO<sub>2</sub>-enriched environments within the physiological range of the maize rhizosphere. Both switching off CO<sub>2</sub> perception and scrubbing plant-associated CO<sub>2</sub> had no effect on the ability of WCR larvae to locate host plants at short distances (<9 cm), but impaired host location at greater distances. The authors conclude that insects use this signal as a long-range cue to orient themselves, but then switch to other odorous cues when closer to their

Arce CCM, Theepan V, Schimmel BCJ, Jaffuel G, Erb M, Machado RAR 2021. Plant-associated CO<sub>2</sub> mediates long-distance host location and foraging behaviour of a root herbivore. *eLife* 10: e65575. DOI: [10.7554/eLife.65575](https://doi.org/10.7554/eLife.65575)



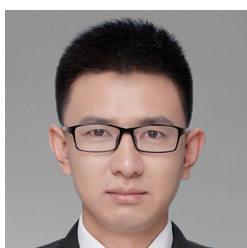
**Plant-associated CO<sub>2</sub> mediates host location by western corn rootworm larvae, either wild type or silenced for CO<sub>2</sub> perception, in a distance-specific manner.** CO<sub>2</sub> scrubber olfactometers were outfitted with soda lime to suppress CO<sub>2</sub> diffusion while allowing for the diffusion of other volatiles. Mean CO<sub>2</sub> concentrations (in ppm) in each olfactometer arm are given inside the bars, and the difference between them to the right. \*\* p<0.01.

target to narrow in on an actual food source. They also found evidence for CO<sub>2</sub> helping the insects find the most suitable roots to feed on, e.g. from plants that grow in soils rich in nutrients and thereby release more carbon dioxide.

## Stealing genes



Zhaojiang Guo



Jixing Xia



Cell cover



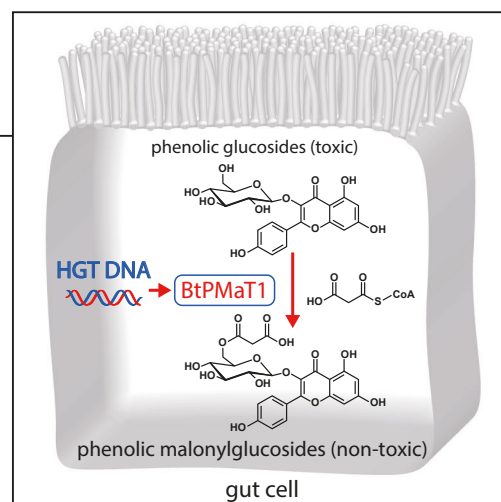
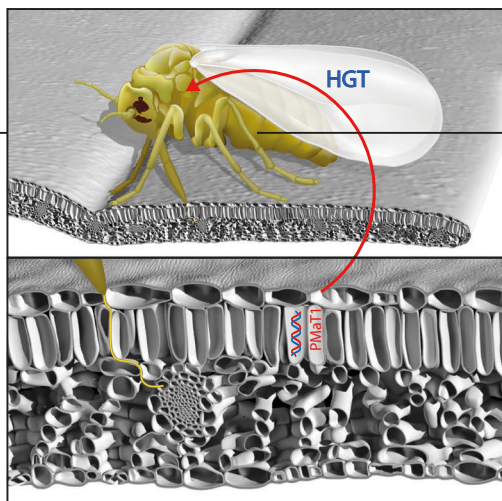
Plant (example: Tomato)

Another collaboration between Ted Turlings and a Chinese research group has culminated in a widely noted publication. The investigation was carried out at the Chinese Academy of Agricultural Sciences in Beijing, to which most of the researchers, including the two first authors Jixing Xia and Zhaojiang Guo are affiliated. The paper provides conclusive evidence for a rare plant-to-insect horizontal gene transfer (HGT) event that enables the receiver, a sap-sucking insect, to detoxify common plant defence compounds.

The cosmopolitan whitefly *Bemisia tabaci* is an extremely polyphagous insect and attacks a wide range of crops whose yield it reduces by feeding on phloem, transmitting plant viruses and excreting honeydew. Using bioinformatic, molecular and biochemical methods, in combination with insect performance assays, the authors demonstrated that the whitefly genome harbours a plant-specific and horizontally transferred gene, *BtPMT1*, that allows the insect to neutralize phenolic glycosides, widespread plant defence compounds. The gene must have been acquired from an unknown plant donor – supposedly via a virus vector – up to 86 million years ago, the time when *Bemisia* diverged from *Trialeurodes*, another whitefly

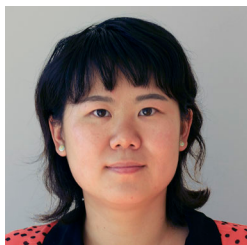
genus which does not carry the gene. *BtPMT1* is expressed in all developmental stages and tissues of *B. tabaci*, but expression is especially high in the gut of adults, which indicates that it plays a key role in detoxification processes. The encoded enzyme glucoside malonyltransferase turns harmful phenolic glycosides into phenolic malonylglucosides innocuous for the whitefly. The stolen *BtPMT1* gene is another weapon in the whitefly's arsenal to overcome the defences of its host plants and a further explanation for why it is such a successful pest all over the world. The study also proposes means to exploit the discovery in pest control: tomato plants genetically transformed to produce small interfering RNAs that silence *BtPMT1* and thus impair the whitefly's detoxification ability were found highly resistant to *B. tabaci*, while non-target organisms were not affected.

The paper was chosen to be featured in a preview article in *Cell* and a spotlight article in *Trends in Genetics*. It has drawn enormous media attention all over the world, but also stirred a bit of controversy because the authors failed to cite two relevant references in the originally published version, an issue that they have rectified in the meantime, both in a [corrigendum](#) and in the main document.



Xia J, Guo Z, Yang Z, Han H, Wang S, Xu H, Yang X, Yang F, Wu Q, Xie W, Zhou X, Dermauw W, **Turlings TCJ**, Zhang Y 2021. Whitefly hijacks a plant detoxification gene that neutralizes plant toxins. *Cell* 184: 1693–1705. DOI: [10.1016/j.cell.2021.02.014](https://doi.org/10.1016/j.cell.2021.02.014)

## Insight into the defensive function of volatiles from the evolution of an invasive plant



Tiantian Lin



Diane Laplanche

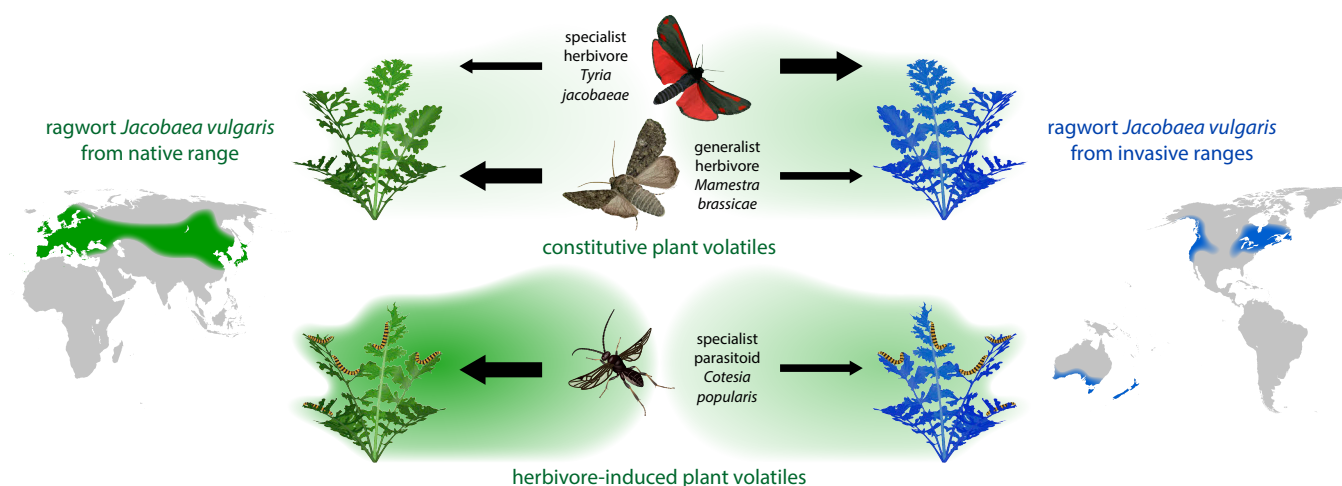


Gaylord Desurmont

There is still an ongoing debate on whether indirect plant defence through recruitment of “bodyguards”, predators and/or parasitoids, is one of the reasons for the evolution of herbivore-induced volatile emissions. A joint study on an invasive plant by the Farce group and researchers at the University of Leiden, suggests that evolutionary changes in the release of constitutive and inducible odours by the plant in its invasive ranges provide further support in favour of such a defensive function. Tiantian Lin, the first author, visited UniNE in 2016 to carry out the required laboratory experiments with the assistance of Diane Laplanche. They collected and analysed the volatiles from different plant populations and conducted behavioural assays with insects in a four-arm olfactometer, whereas the field work was conducted in the Netherlands.

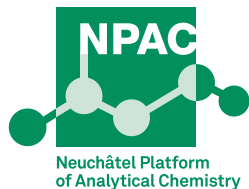
More than a hundred years ago, ragwort *Jacobaea vulgaris*, native to Eurasia, invaded North America, Australia and New Zealand, and it thrived there until recently in the absence of its associated specialist herbivore, the cinnabar moth *Tyria jacobaeae*. Plants from invasive populations were found to release higher levels of constitutive volatiles, but considerably lower levels of herbivore-induced volatiles than

native plants. As theory predicts, undamaged plants from the invasive ranges proved more attractive to females of the specialist cinnamon moth than plants from the native ranges, while the opposite was observed for females of the generalist cabbage moth *Mamestra brassicae*. The respective oviposition preferences of both species followed the same pattern. Also as expected, the higher amounts of volatiles emitted by native plants under attack of *Tyria* caterpillars attracted more *Cotesia popularis* parasitoids, a species specialized on this herbivore. This was reflected in higher parasitism rates recorded for caterpillars feeding on native plants when grown alongside invasive plants in a field trial in the Netherlands. The changes in volatile emissions went in the same direction in the three invasive areas in spite of the distinct climatic conditions. Hence, they were not attributed to abiotic factors, but rather to the release from herbivore pressure. The study adds another piece to the mosaic picture in favour of the hypothesis that the evolution of foliar volatile emissions is driven by their direct and indirect roles in defence against insects. The relevance of these findings was highlighted by a [dispatch commentary](#) published in the same issue of *Current Biology*.



Lin T, Vrieling K, Laplanche D, Klinkhamer PGL, Lou Y, Bekooy L, Degen T, Bustos-Segura C, Turlings TCJ, Desurmont GA 2021. Evolutionary changes in an invasive plant support the defensive role of plant volatiles. *Current Biology* 31: 3450-3456. DOI: [10.1016/j.cub.2021.05.055](https://doi.org/10.1016/j.cub.2021.05.055)

## Metabolomics rules!



<https://www.unine.ch/npac>

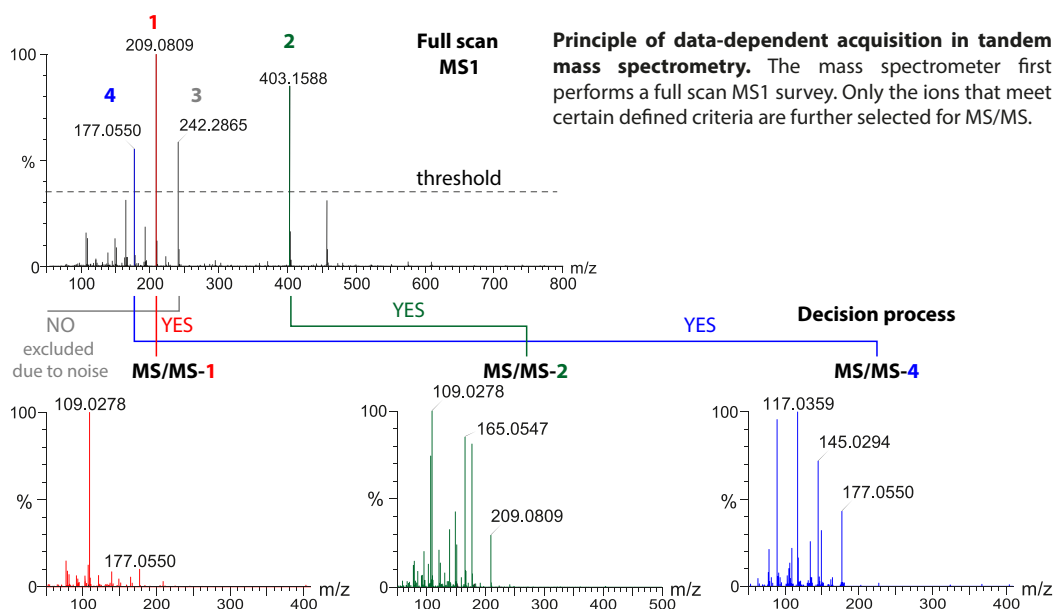


Gaétan Glauser

Metabolomics has become a central approach for the holistic analysis of metabolites in biological systems. The underlying technology keeps on getting more and more powerful and sophisticated, which is necessary to deal with the huge amounts of acquired data. This opens new opportunities for improved metabolomics workflows, but also makes the development of optimal analytical methods to exhaustively detect, characterize, and quantify the entire metabolome quite a challenge.

Tandem mass spectrometry (MS/MS) is one of the preferred methods for metabolomics. One complex challenge in MS/MS-based metabolomics, however, is to combine efficient screening capacity for enhanced metabolome coverage with qualitative spectral fragmentation for improved marker identification. Among the available workflows for untargeted metabolomics, data-dependent acquisition (DDA) offers the possibility to acquire clean MS/MS spectra, based on real-time evaluation

of MS data by the instrument software. In the course of their investigations on DDA with the Waters SYNAPT XS mass spectrometer, the new addition to the NPACs arsenal that we presented in the previous C<sub>3</sub>E Newsletter, researchers at the NPAC and the Functional Ecology laboratory realized that many of the DDA-based metabolomics methods published in the literature used idiosyncratic parameters with a poorly defined rationale behind. They thus decided to write a review, which provides recommendations on how to choose and optimize the technical parameters essential to perform DDA metabolomics experiments, such as cycle time, threshold for switching to MS/MS, use of inclusion and exclusion lists, isolation window for precursor selection etc. The proposed rules should help users make appropriate decisions when setting up a DDA method and ensure proper data acquisition for further processing workflows for the metabolomics community.



Defossez E, Bourquin J, Rasmann S, von Reuss S, Glauser G 2021. Eight key rules for successful data-dependent acquisition in mass spectrometry-based metabolomics. *Mass Spectrometry Reviews*: 1- 13. DOI: [10.1002/mas.21715](https://doi.org/10.1002/mas.21715)

## Uncovering the ecological secrets of the three sisters



Betty Benrey

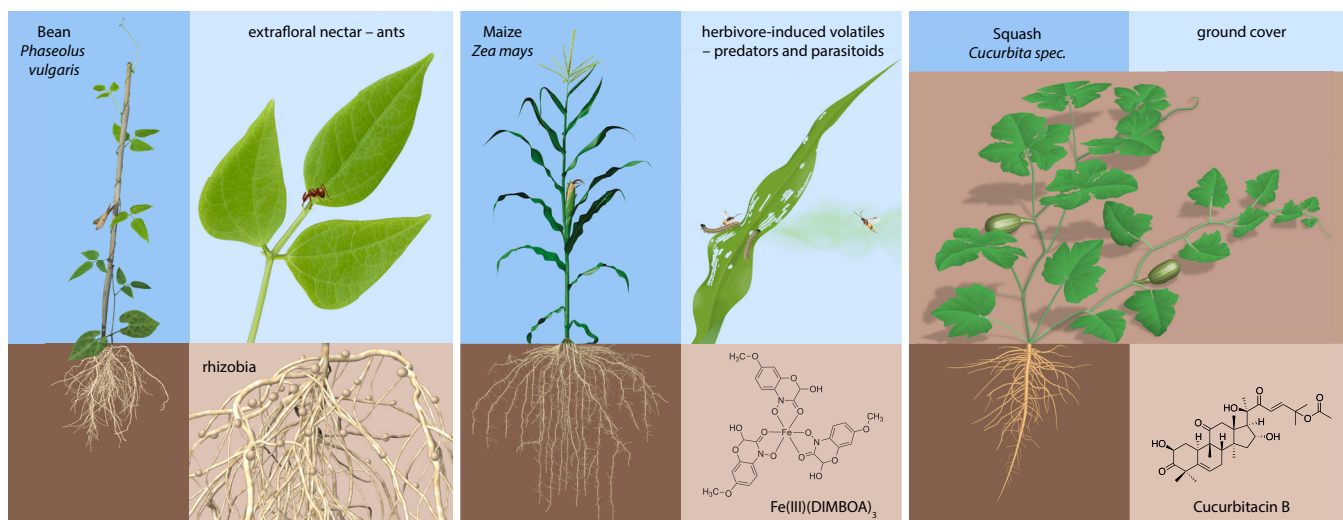


Patrick Grof-Tisza

Betty Benrey has been awarded a grant by the Swiss National Science Foundation, which allows her to study the ecological mechanisms underlying the success of a traditional crop-growing system practiced in her home country Mexico. The so-called Milpa has proven highly productive and relatively invulnerable to pests. It combines the cultivation of **three principal crops** aka the three sisters that are considered to interact in a mutualistic way: **bean plants** produce extrafloral nectar, which can attract ants and other potential “bodyguards” for protection against herbivores. Their root nodules with nitrogen-fixing rhizobia enhance the nutritional quality of the soil. **Maize plants** provide shade and support for the vines of bean plants, but also possess mutually beneficial chemical traits. Upon herbivore attack, they release large amounts of volatiles attracting the natural enemies of the herbivores. Their roots exudate benzoxazinoids, which are toxic to non-specialist soil pests, but also facilitate iron transport and uptake by forming complexes. **Squash plants** cover the ground, preventing

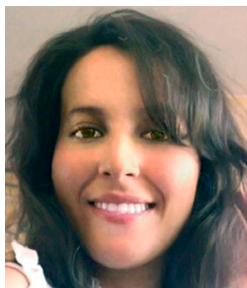
the establishment of weeds and maintaining high levels of soil moisture. The cucurbitacins released by their roots have allelopathic effects and deter generalist herbivores.

The researchers will test the hypothesis that the combination of these plant traits drives the mutualistic benefits that the Milpa plants derive from each other. They will address the following question: Do the combined plant-mediated effects on soil conditions and the direct and indirect plant defence traits enhance plant performance in a mutually beneficial manner? A postdoctoral fellow, Patrick Grof-Tisza, together with a PhD student, Camilo Rivera, will conduct a combination of laboratory, field cage and open field experiments, involving behavioural, performance, molecular and metabolomics assays. The field work will be carried out in the state of Oaxaca, Mexico, where Milpa is commonly practiced. It is hoped that the insight into the ecological processes underlying this successful traditional agroecosystem may lead to an improvement of sustainable pest management strategies.



<b>Title</b>	<b>Unraveling the ecology behind the success of traditional Milpa intercropping in Mesoamerica</b>
Funding Organization	Swiss National Science Foundation
<b>Funding Scheme</b>	<b>Project funding in biology and medicine (division III)</b>
Budget	526'667 CHF
Start/End	1.10.2020 - 31.1.2024

## Connecting lipogenesis and ascaroside biosynthesis



Hanna Laaroussi

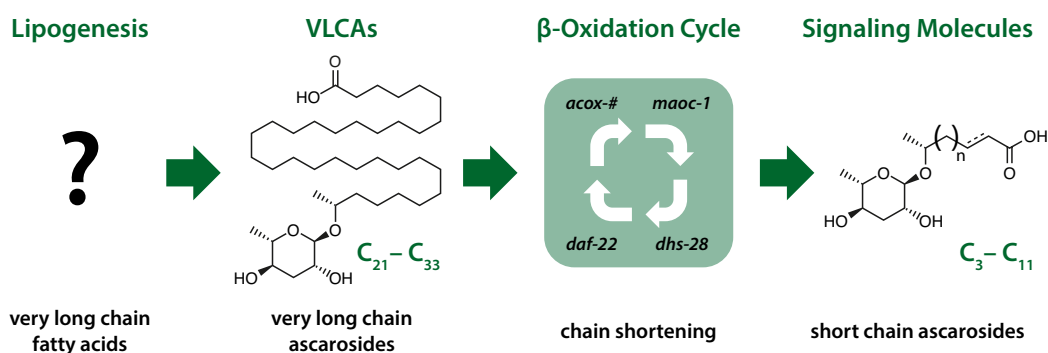


Stephan von Reuss

Stephan von Reuss, head of the Laboratory for Bioanalytical Chemistry, has launched a new project funded by the Swiss National Science Foundation to study as yet unresolved questions concerning the biosynthesis of ascarosides, a diverse group of glycolipids in nematodes that modulates development, behaviour and interactions with other organisms. The investigation will be conducted with the bacterivorous nematode *Caenorhabditis elegans*, an extremely well-studied model organism in developmental biology and genetics.

Hanna Laaroussi, who has received a Master's Degree in Molecular Pharmacology from the Université de Paris, has joined Stephan's team as a PhD student. Hanna's research involves the total synthesis of stable isotope labelled putative intermediates in ascaroside biosynthesis and their application in feeding experiments in order to decipher the molecular basis of the biosynthetic steps that connect lipogenesis and ascaroside biosynthesis. Short-chain ascarosides endowed with diverse signalling functions are produced via repetitive chain shortening of very long chain

ascaroside (VLCAs) intermediates, thereby depending on a primary metabolic pathway, the peroxisomal  $\beta$ -oxidation cycle of fatty acid metabolism. The biosynthetic origin of the VLCAs upstream of the  $\beta$ -oxidation cycle has remained largely unknown. Preliminary results from a mixed isotope labelling experiment suggested the presence of at least three segregated lipogenic pathways that contribute to VLCA biogenesis. To elucidate the linkage between lipogenesis and ascaroside biosynthesis, the researchers will combine natural product analysis including isolation and structure elucidation with total synthesis to identify biosynthetic intermediates and the order of their transformations. Furthermore, the project aims at identifying yet uncharacterized genes that are involved in the biosynthetic steps that connect lipogenesis and ascaroside biosynthesis. To achieve this goal, a combination of reverse genetics with deletion mutants and gene silencing by RNAi coupled to selective mass spectrometric ascaroside screens will be employed by another PhD student or a post-doctoral researcher.



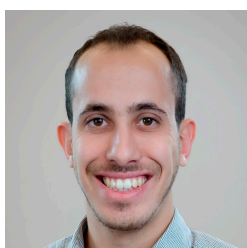
Schematic representation of ascaroside biogenesis including the chain shortening of very long chain ascarosides by peroxisomal  $\beta$ -oxidation resulting in the short chain ascarosides with signalling function.

<b>Title</b>	<b>Linking lipogenesis and ascaroside signaling in the model organism <i>Caenorhabditis elegans</i></b>
<b>Funding Organization</b>	Swiss National Science Foundation
<b>Funding Scheme</b>	<b>Project funding in mathematics, natural and engineering sciences (division II)</b>
<b>Budget</b>	651'322 CHF
<b>Start/End</b>	1.2.2021 - 31.1.2025

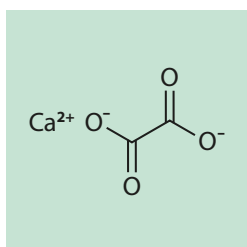
## A BRIDGE project for troubled lungs



Pilar Junier



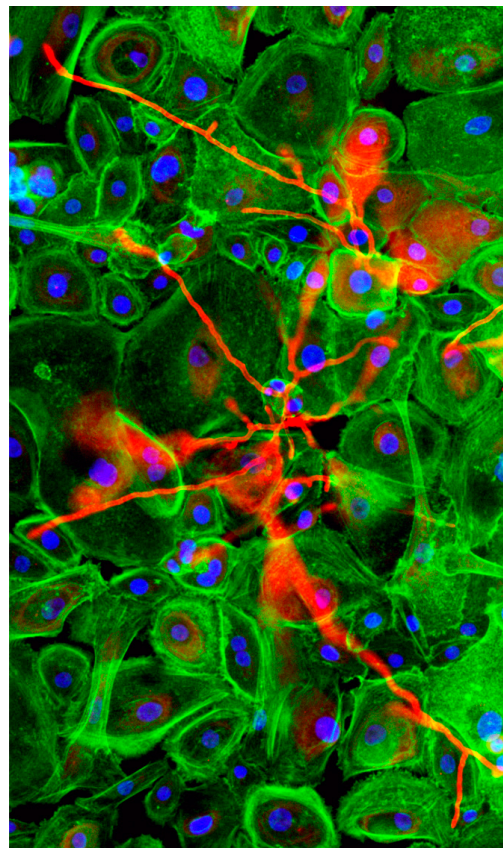
Fabio Palmieri



Calcium oxalate

Pilar Junier has obtained a prestigious *BRIDGE discovery* grant for a joint project with Angela Koutsokera (Lausanne University Hospital – CHUV) and Marco Pagni (Swiss Institute of Bioinformatics – SIB). The *BRIDGE* funding scheme is a joint programme conducted by the Swiss National Science Foundation and Inno-suisse - the Swiss Innovation Agency offering funding opportunities at the intersection of basic research and science-based innovation. *Discovery* is aimed at experienced researchers who intend to explore and implement the innovation potential of research results expected to have a societal and economic impact.

Despite the fact that every year an estimated number of 1.5 million people die of fungal infections, health problems caused by these pathogens tend to be neglected. There is only a limited number of antifungal drugs available for treatment. These also tend to become less efficient due to resistance, favoured by the fact that they are often used in both agriculture and animal and human health. In addition, the matter has attained actuality because the lungs of SARS-CoV-2 patients are supposed to be more susceptible for secondary infections by fungal pathogens like *Aspergillus*. The project examines a new approach to treat pulmonary aspergillosis, which can be denoted as “environmental interference”, aiming to change lung ecology in a way unfavourable for fungal growth. *Aspergillus* fungi secrete oxalic acid, thereby decreasing pH and forming calcium oxalate crystals. The strategy now consists in counteracting this effect by promoting oxalotrophic bacteria from the lung microbiota that re-establish physiological pH and free calcium concentrations. Fabio Palmieri from Pilar Junier’s microbiology group could show in his PhD thesis that this principle works in a lung-on-a-chip system. These promising results



Fluorescence microscopy image of bronchial epithelial cells infected by a fungus (*Aspergillus*, Actin, DNA). Picture by Fabio Palmieri.

shall now be translated into an in vivo murine model to validate the clinical relevance of the method. The researchers also intend to screen for biocontrol airway bacteria suitable for transplantation of oxalotrophy into lung microbiota that lack this function. These and further goals are to be achieved through an academic-industrial partnership, including also clinicians at the hospital and two pioneering Swiss SMEs working in the field of cell culture and organ-on-chip technologies.

<b>Title</b>	<b>CaOx: applying ecological theory in the fight against lung fungal pathogens</b>
Funding Organization	Swiss National Science Foundation
<b>Funding Scheme</b>	<b>Bridge - Discovery</b>
Budget	1'800'000 CHF
Start/End	1.3.2021 - 28.2.2025