

# NSABS 2020

25<sup>th</sup> National Symposium for Applied Biological Sciences

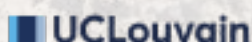
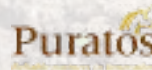
January 31<sup>st</sup> 2020

Gembloux Agro-Bio Tech - Belgium

Espace Senghor - Avenue de la Faculté 5030 Gembloux

Plant science  
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Forestry  
Agriculture  
Management  
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→ <https://www.gembloux.ulg.ac.be/nsabs-2020/> ←



## Thank you to the NSABS 25th annual symposium sponsors and partners



# Table of contents

Click on the  
page number



NSABS .....	p. 4
Welcome to Gembloux Agro-Bio Tech .....	p.5
Organizing committee .....	p.6
The city of Gembloux .....	p.7
Conference venue .....	p.8
Information for participants .....	p.9
NSABS meeting tries to be sustainable .....	p.10
<b>Program</b> .....	p.11
Crop & livestock precision farming .....	p.12
Food technology, safety & health .....	p.30
Water & soil science .....	p.59
Sustainable crop production & protection .....	p.77
Biotechnology .....	p.106
Climate change .....	p.142
Urban ecosystem, Forest ecology & management .....	p.160
Green chemistry & bio-products .....	p.180

# NSABS

## 25th National Symposium for Applied Biological Sciences (NSABS)

NSABS 2020 bring together junior researchers, postdocs and group leaders in the field of applied biological sciences.

The full-day symposium will encompass a wide variety of research topics in applied biological sciences, and particularly encourages PhD students to present their work, meet and exchange ideas across researchers and institutions.

Prizes will be awarded for the best oral and poster presentations.



## Previous editions

[NSABS 2019](#)

[NSABS 2018](#)

[NSABS 2017](#)

# Welcome to Gembloux Agro-Bio Tech

Gembloux Agro-Bio Tech, a faculty of the University of Liège, exclusively dedicated to life sciences and bioengineering.

For more than 150 years, Gembloux Agro-Bio Tech has established itself as a true centre of expertise in the field of life sciences and biological engineering.

Integrated into the University of Liège since the 1st of October 2009, Gembloux Agro-Bio Tech is a friendly-sized faculty recognised internationally for the quality of its teaching and the excellence of its research. This reputation attracts more than a thousand students from some forty countries.

Gembloux Agro-Bio Tech trains academics and engineers to be able to meet the challenges facing society in the fields of environmental protection, production, processing and enhancement of bioresources.

The Faculty of Gembloux Agro-Bio Tech is internationally recognized for the quality of its teaching and research.

Four bioscience engineering master's degrees are offered to students, enabling them to specialize in key areas of life sciences: environmental sciences and technologies, forest and natural space management, agronomic sciences and chemistry and bio-industries.

These specializations prepare students to manage technological transition, to use biotechnologies, agro-materials and biofuels, manage natural resources (water, soil, air, etc.), to purify, depollute, and develop new medicines and new forms of food, etc.

In collaboration with other faculties of the University of Liège and partner institutions, Gembloux Agro-Bio Tech also organizes comprehensive training in landscape architecture, and offers a master's degree in the following areas: agroecology innovation management and food design, and integrated production and preservation of natural resources in urban and peri-urban areas.

Gembloux Agro-Bio Tech's research and development, as well as its concerns of issues of international and societal interest, makes it a key player in sustainable development.

One of the priorities of Gembloux Agro-Bio Tech is to increase scientific research both quantitatively and qualitatively. That is why it has set up the brand-new TERRA research centre.

The Faculty of Gembloux Agro-Bio Tech also fulfils the mission of « service to society ». Thanks to its specialized expertise, the Office of Environment and Analysis (BEAGx) in Gembloux provides assistance to the general public, professionals and companies in matters of environment, pollution and quality control.



# Organizing committee

## Organizing committee

Hélène Soyeurt [coordinateur] (ULiège)  
Dominique Marchal [secrétariat] (ULiège)  
Arnaud Monty (ULiège)  
Martine Schroyen (ULiège)  
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Jeroen Meersmans (ULiège)  
Bram Van Bergh (KULeuven)  
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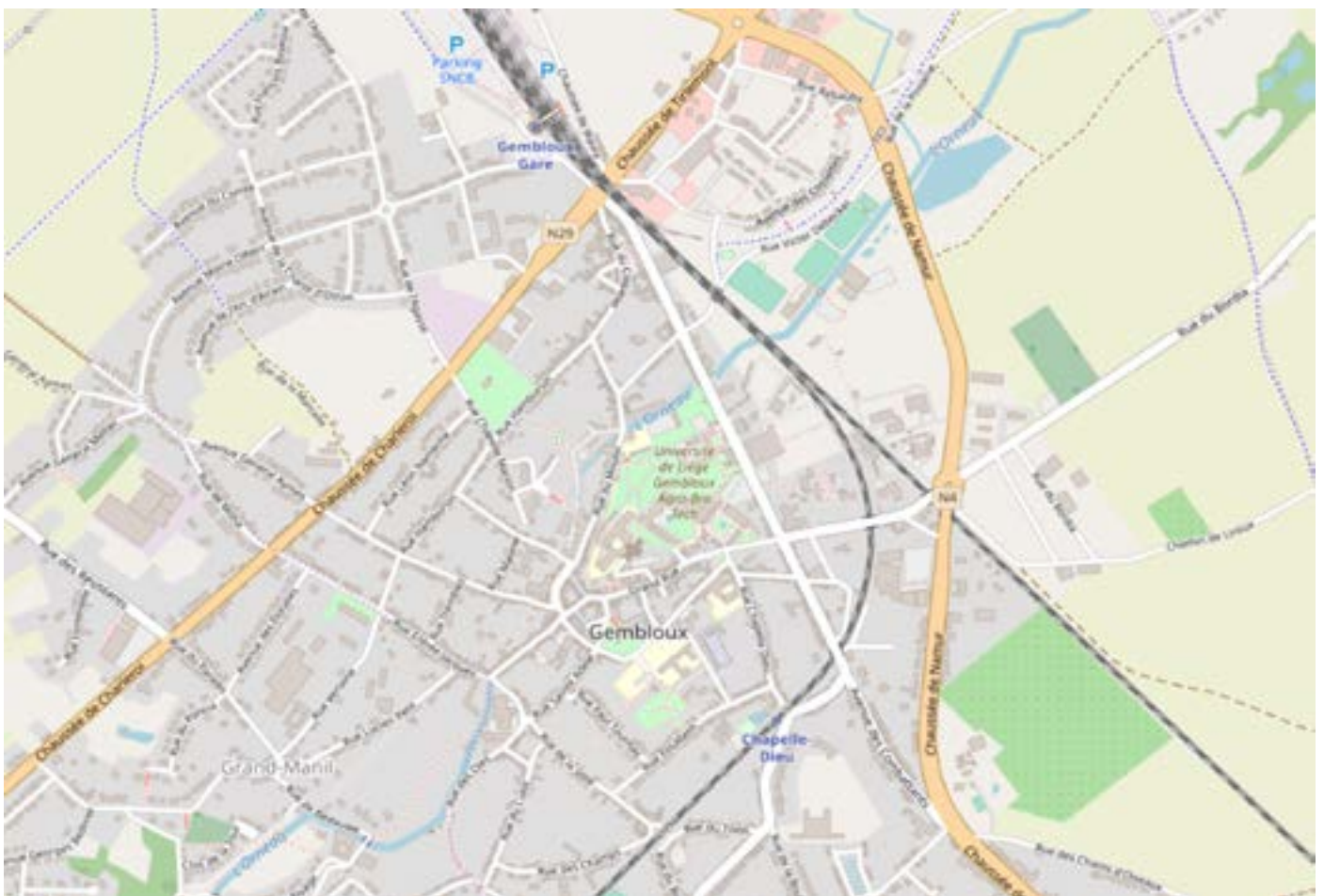
# The city of Gembloux

Gembloux is a small town of just over 25,000 inhabitants located in French-speaking Belgium in the province of Namur.

It is a historic city, as evidenced by the Benedictine abbey that today houses the Faculty of Gembloux Agro-Bio Tech and the belfry of which has been listed as a UNESCO World Heritage Site.

The city of Gembloux is centrally located in Belgium and Europe which makes it easily accessible by public transport (plane, train, bus).

Living in Gembloux also means enjoying the tranquil setting of a cosy city.



# Conference venue

The conference will take place in the Espace Senghor or BV or ZT1, Gembloux

## **Espace Senghor + parking**

Entrée 7 - Quartier Senghor

Avenue de la Faculté d'Agronomie, 11

50,56 30 13 Latitude Nord - 4,69 75 72 Longitude Est

## **Entrées BOTANIQUE**

Entrée 2 - Quartier BOTANIQUE

Avenue Maréchal Juin, 13

50,56 20 45 Latitude Nord - 4,70 12 02 Longitude Est

Entrée 3 - Quartier BOTANIQUE

Avenue Maréchal Juin, 6

50,56 18 32 Latitude Nord - 4,70 03 94 Longitude Est

Entrée 4 - Quartier BOTANIQUE

Avenue Maréchal Juin, 13

50,56 18 67 Latitude Nord - 4,69 98 76 Longitude Est

## **Entrée Cour d'honneur**

Entrée 5 - Quartier Palais

Passage des Déportés, 2

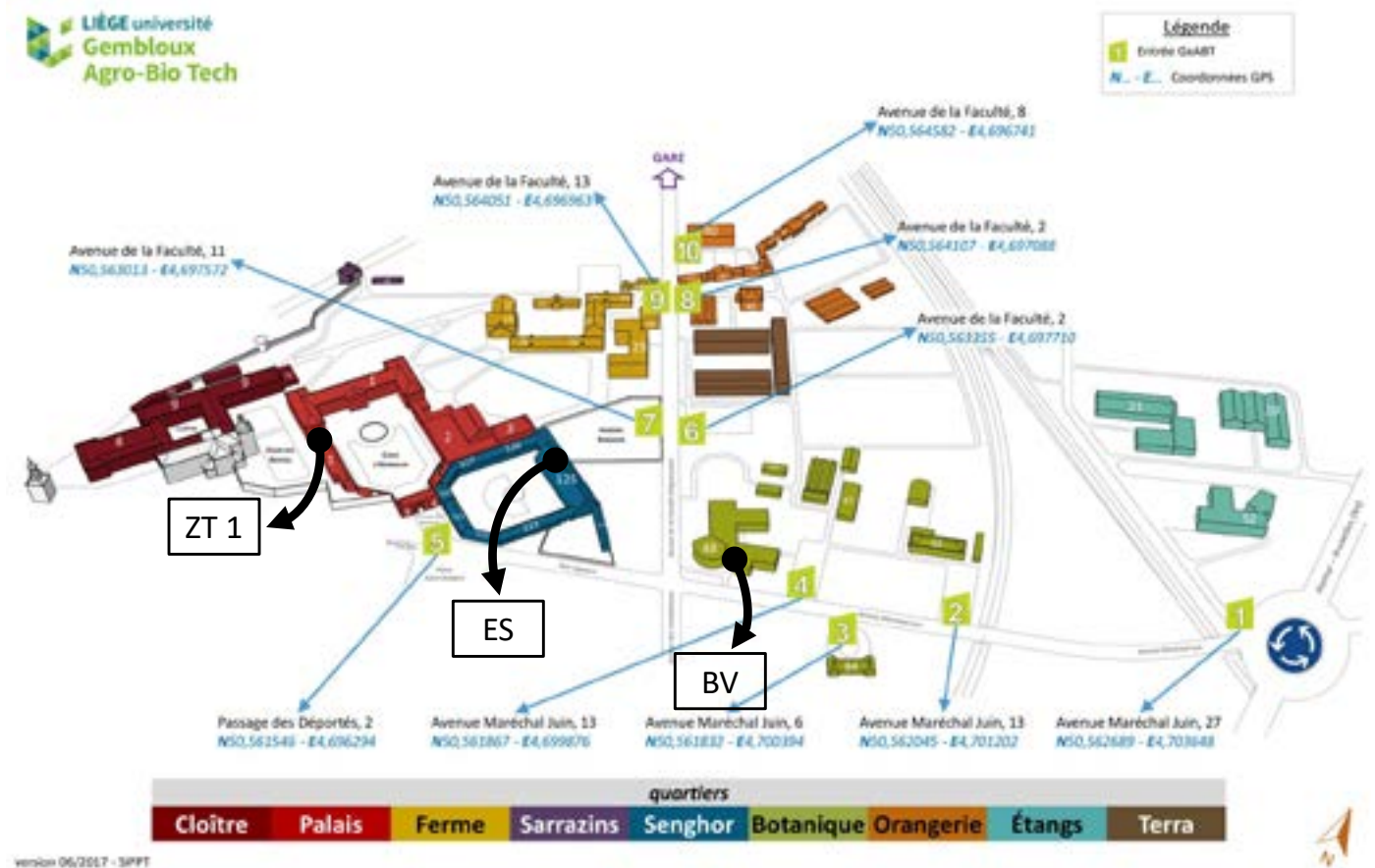
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# Information for participants

## Auditorium

Espace Senghor (ES)  
Biologie Végétale (BV)  
Zootechnie 1 (ZT1)



**Conference website :** [www.gembloux.ulg.ac.be/nsabs-2020](http://www.gembloux.ulg.ac.be/nsabs-2020)

**Conference email :** [nsabs2020@uliege.be](mailto:nsabs2020@uliege.be)

## Badges and identification :

Upon registration each participant will receive a name badge.

## Wi-Fi

Visitors can use the free Wi-Fi network. Name of the WIFI network and code will be available at the conference center.

# NSABS meeting tries to be sustainable

The catering is local (see the map) and as much as possible seasonal.

We have limited the number of printed papers.

The dishes are reusable.

**... Enjoy your meeting ...**



# Program

**8:30 – 9:00** – Registration, coffee and poster fixing (ES)

**9:00 – 9:15** – Welcome Dean of Gembloux Agro-Bio Tech + Prof. dr. ir. Hélène Soyeurt (ES)

**9:15 – 10:00** – “The researchers come from Mars, the journalists from Venus”, François Louis, RTBF journalist (ES)

**10:00 – 10:45** – “Reconnecting agriculture to food systems for global sustainability: the example of long term experimental crop rotations of AgricultureIsLife”, Prof. Jérôme Bindelle, ULiège (ES)

**10:45 – 11:15** – Coffee break + poster session (ES)

**11:15 – 12:30** – 3 parallel sessions + flash talks

*Crop & livestock precision farming (ES)*

*Food technology, safety & health (BV)*

*Water & soil science (ZT1)*

**12:30 – 13:30** – Lunch break + Poster session (ES)

**13:30 – 14:45** – 3 parallel sessions + flash talks

*Sustainable crop production & protection (ES)*

*Biotechnology (BV)*

*Climate change (ZT1)*

**14:45 – 15:15** – Coffee break + Poster session (ES)

**15:15 – 16:30** – 3 parallel sessions + flash talks

*Urban ecosystem, Forest ecology & management (BV)*

*Green chemistry & bio-products (ES)*

**16:30 – 17:00** – Closing remarks and awards (ES)

**17:00** – Reception (ES)

Session 1 (ES)

# Crop & livestock precision farming

11h15 -12h15

**Chairman: Jan Baetens – UGent**

Towards a compartment-based growth-quality model of tomato (*Solanum Lycopersicum* cv. Merlice): fruit growth modelling  
Jakub Šalagovič (KULeuven)

Strategies of the Walloon dairy breeders faced to the uncertain dairy future  
Anne-Catherine Dalcq (ULiège)

Improve quality of free-range eggs by choosing vegetal species of grassland  
Victoria Tosar (CRA-W)

Added value of plant height and multi-temporal modelling to improve the estimation of winter wheat, *Triticum aestivum* L., above-ground traits based on proxy-sensing RGB cameras  
Sébastien Dandrifosse (ULiège)

Flash presentations

## DIFFERENTIAL EFFECTS OF SULFATE AND CHLORIDE SALINITIES ON RICE GENES EXPRESSION PATTERNS: A COMPARATIVE TRANSCRIPTOMIC APPROACH

Willy Irakoze<sup>1</sup>, Gervais Rufyikiri<sup>2</sup>, Muriel Quinet<sup>1</sup>, Stanley Lutts<sup>1</sup>

<sup>1</sup> *UCLouvain, Belgium*

<sup>2</sup> *Geneva Centre for Security Policy (GCSP), Switzerland*

Salinity is among the most serious challenges to rice production in the world today. To elucidate genome-level responses to chloride or sulfate salinity stress in rice, I Kong Pao (salt-sensitive cultivar) seedlings have been exposed to salt stress (EC of c.a 20 dS m<sup>-1</sup> NaCl or Na<sub>2</sub>SO<sub>4</sub>) for 48 hours. A combined transcriptomic and physiological study have been performed after exposure to saline solutions in order to clear the main metabolic pathways involved in rice response to salt stress. Our results showed that a large number of genes were expressed under sulfate than chloride salinity, this difference being the most remarkable in root. Most of the genes involved in response to salt stress were up-regulated in Na<sub>2</sub>SO<sub>4</sub>-treated plants whatever the considered organs while more genes were down-regulated in NaCl-treated plants, organs combined. The comparison of expression profiles among different salinity stresses showed largely stress-specific patterns of regulation. Moreover, both stresses appeared to alter the expression patterns of a significant number of genes involved in response to abiotic stimulus, signal transduction and transport in a largely organ-specific manner. Modification in some genes expression could be followed by modification in corresponding metabolic products and physiological properties, or differed depending on the type of salt stress or organ for some others, underlying the importance of an integrated study. Our study offered the first comprehensive picture of genome expression modulation in response to sulfate salinity stress in two distinct rice organs and could be very useful in improving of rice plant tolerance to Na<sub>2</sub>SO<sub>4</sub> stress.

## Towards a compartment-based growth-quality model of tomato (*Solanum Lycopersicum* cv. Merlice): fruit growth modelling

Jakub Šalagovič,<sup>\*</sup> Inez Driesen<sup>1</sup>, Maarten Hertog<sup>1</sup>, Bert Verlinden<sup>2</sup>, Pieter Verboven<sup>1</sup>, Bram Van de Poel<sup>1</sup>, Kristof Holsteens<sup>1</sup>, Marlies Huysmans<sup>3</sup>, Bart Nicolai<sup>1,2</sup>

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<sup>2</sup>VCBT, Leuven, Belgium

<sup>3</sup>Proefcentrum Hoogstraten, Meerle, Belgium

Tomato is together with grape the best known model fruit for fruit growth [1]. Different approaches for tomato fruit development modelling exist – from crop growth models, the first ones able to predict practical characteristics such as yield [2, 3], through biophysical mechanistic models [4-6] to virtual fruit models [7]. However, to our knowledge there is currently no mechanistic model for plant and fruit growth and quality change from cell division to ripening stage.

To achieve such a model, we proposed using a compartment model structure, first introduced in the GreenLab model [8]. This modeling approach is situated in between process-based models and functional-structural plant models. It contains individual fruits and source-sink competition to increase preciseness of the model and to get better insight into biophysical processes behind it. The model was divided into compartments based on organ type, age and position in the plant. Each compartment was modeled as a population of respective organs using the process-based approach. Interaction between compartments and making abstraction of individual fruits and their topology was inherited from functional-structural plant models. This allowed us to use different environmental settings for different parts of a plant while still keeping complexity of the model low compared to the functional-structural plant approach.

As a first step, the modelling of fruit growth of individual tomatoes was adapted and validated against experimental data from different climate conditions, focusing on temperature differences. Both experiments and the model showed a higher effect of temperature during early stages of fruit development compared to later stages.

### Acknowledgements

The research was partially funded by the Interreg Vlaanderen-Nederland GROW! project.

### References

- [1] CLEARWATER, Michael J., et al. Vascular functioning and the water balance of ripening kiwifruit (*Actinidia chinensis*) berries. *Journal of Experimental Botany*, 2011, 63.5: 1835-1847.
- [2] HEUVELINK, Egbert. *Tomato growth and yield: quantitative analysis and synthesis*. Heuvelink, 1996.

- [3] JONES, James W., et al. A dynamic tomato growth and yield model (TOMGRO). *Transactions of the ASAE*, 1991, 34.2: 663-0672.
- [4] FISHMAN, S.; GÉNARD, M. A biophysical model of fruit growth: simulation of seasonal and diurnal dynamics of mass. *Plant, Cell & Environment*, 1998, 21.8: 739-752.
- [5] HALL, Alistair J., et al. A biophysical model of kiwifruit (*Actinidia deliciosa*) berry development. *Journal of experimental botany*, 2013, 64.18: 5473-5483.
- [6] LIU, Huai-Feng, et al. Model-assisted analysis of tomato fruit growth in relation to carbon and water fluxes. *Journal of Experimental Botany*, 2007, 58.13: 3567-3580.
- [7] GÉNARD, Michel, et al. Virtual profiling: a new way to analyse phenotypes. *The Plant Journal*, 2010, 62.2: 344-355.
- [8] DE REFFYE, P., et al. Coupling process-based models and plant architectural models: a key issue for simulating crop production. In: *Crop modeling and decision support*. Springer, Berlin, Heidelberg, 2009. p. 130-147.

# Strategies of the Walloon dairy producers faced to the uncertain dairy future

Anne-Catherine Dalcq<sup>1\*</sup>, Thomas Dogot<sup>1</sup>, Hélène Soyeurt<sup>1</sup>, Yves Brostaux<sup>1</sup>, Frédéric Vanwindekens<sup>2</sup>, Eric Froidmont<sup>2</sup>, Pierre Rondia<sup>2</sup>, Benoit Wyzen<sup>3</sup>, Alain Masure<sup>4</sup>, Catherine Bauraind<sup>5</sup> and Yves Beckers<sup>1</sup>

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<sup>2</sup> CRA-W, Rue du Liroux, 8, 5030 Gembloux, Belgium

<sup>3</sup> Eleveo, Rue des Champs Elysées, 4 5590 Ciney, Belgium

<sup>4</sup> FWA, Chaussée de Namur 47, 5030 Gembloux, Belgium

<sup>5</sup> Collège des Producteurs, Avenue Comte de Smet de Nayer 14, 5000 Namur, Belgium

\*[anne-catherine.dalcq@uliege.be](mailto:anne-catherine.dalcq@uliege.be)

This study observes the strategies, and their determinants, of the Walloon dairy producers faced to the post quota perspective through the realisation of 245 surveys, conducted from November 2014 to February 2015. It highlights how dairy production companies plan to evolve to cope with this great change in the sector and so how will move the production of our dairy products. Three kinds of strategical variables were defined and related to the evolution of milk production (**MP**) [the producers who increase MP (**HighMP**) vs. keep constant MP (**ConstantMP**) vs. stop MP]; the valorisation of MP [alternative (**ValMP**) vs. classical] and the diversification of activities [with (**DivMP**) vs. without such activities]. The relationships between the chosen strategies and the quantitative technical variables were studied using generalised linear models. The independence between qualitative technical variables and the strategical variables was tested using Chi Square test. HighMP and ConstantMP producers represent 38.4% and 53.9% of respondents, respectively. HighMP producers were significantly more declared as legal entity (p-value = 0.03), had more family members on the farm (p-value < 0.01), larger agricultural area in property (p-value = 0.03) and higher MP quota (p-value = 0.01) compared to ConstantMP producers. Only 9.8% of respondents decide to valorise differently MP. ValMP producers tend to have more employees (p-value = 0.08) and an agricultural area less fragmented (p-value = 0.07) than classical producers. A total of 7.8% of respondents decide to develop other activities. DivMP producers tend to have more employees (p-value = 0.10), more agricultural area in property (p-value = 0.03) and a more recent year of installation (p-value < 0.01). Finally, 44.9% of ConstantMP producers do not want to start an alternative valorisation of MP and diversify their activities. In conclusion, a relationship exists between, amongst others, the legal status, workforce available, characteristics of the agricultural area, the dairy production and the strategy chosen by the Walloon dairy producers.

## Effect of two fertilizer forms on tomatoes plants grown in hydroponic conditions under different P concentrations

Yousra EL-Mejjaouy<sup>a,b</sup>, Youssef Zeroual<sup>c</sup>, Mohamed EL Gharous<sup>a</sup>, Aziz Soulaïmani<sup>a</sup>, Benoît Mercatoris<sup>b</sup>, Benjamin Dumont<sup>b</sup>, Abdallah Oukarroum<sup>a</sup>

<sup>a</sup>*Mohammed VI Polytechnic University, AgroBioSciences and Fertilization Program, Benguerir 43150, Morocco.*

<sup>b</sup>*Liege University, Gembloux Agro Bio-Tech Faculty 5030, Belgium*

<sup>c</sup>*Groupe OCP*

Phosphorus (P) is the second most important nutrient in plant growth and act as limiting factor in agricultural production. Therefore, establishing an accurate, fast, and operable method for diagnosing crop nutrition is very important for crop nutrient management. In this regard, a hydroponic trial was conducted in order to understand P-uptake and the effectiveness of two forms of fertilizers (MAP and NPK) combined with three concentration levels of phosphorus (15, 31 and 60 ppm) and to evaluate the plant physiological response. Physiological parameters measured include chlorophyll content, the change of the chlorophyll (Chl) *a* fluorescence transient, Chl *a* fluorescence parameters, stomatal conductance, as well as dry weight of shoots, roots and stems on hydroponically grown tomato. The ANOVA analysis did not show significant effect of concentration level and fertilizer form on the stomatal conductance. On one hand, the level of P and fertilizer form did not affect dry weight of roots, shoots and stems. On the other hand, chlorophyll content index increased significantly with NPK form compared to MAP form. 11 days after treatments, a significant effect of P concentration level was found, the two levels of P (60 and 31 ppm) were found to have higher values of chlorophyll content than 15 ppm P concentration.

Keywords: Phosphorus, hydroponics, Chlorophyll fluorescence, use efficiency.

1 Improve quality of free-range eggs by choosing vegetal species of grassland

2

3 Tosar Victoria<sup>1</sup>, Rousseau Gilles<sup>1</sup>, Larondelle Yvan<sup>2</sup>, Froidmont Eric<sup>1</sup>

4 <sup>1</sup>Walloon Agricultural Research Center

5 <sup>2</sup>Faculty of Bioscience Engineering AGRO Louvain

6

7 Egg, the worldwide most consumed animal product, shows a high nutritional value and its  
8 composition is easy to improve by modifying laying hens' diet. Equol, a bacterial metabolite derived  
9 from the transformation of isoflavones (daidzein, formononetin) by digestive tract bacteria, is  
10 associated with a wide panel of benefits on human health. Whereas less than 30 % of western people  
11 are able to produce it, producing equol enriched eggs would be relevant for health consumers'. The  
12 aim of this study is to evaluate the production of equol enriched eggs in outdoor conditions. 17-  
13 weeks-old laying hens were distributed on eight outdoor parcels, sown with a single vegetal specie.  
14 Four species (repeated twice) were tested: ryegrass, red clover, chicory and white clover. Eggs were  
15 daily collected, counted and weighed for each henhouse. Mean laying rate was  $87 \pm 10$  % and mean  
16 egg weight was  $60 \pm 5$  g. These performance parameters were not significantly different between  
17 vegetal species. Grasses and eggs were sampled each fortnight to evaluate seasonal evolution of  
18 isoflavones and equol concentrations. In grass, significant differences were observed between  
19 vegetal species, with higher concentrations in red clover. Equol concentrations in egg yolk was also  
20 higher with red clover treatment ( $1155 \pm 76$  ng/g DM) than with other treatments (lower than 300  
21 ng/g DM). The ingestion of one egg could provide about 1 µg of equol, which seems to be a  
22 significative quantity.

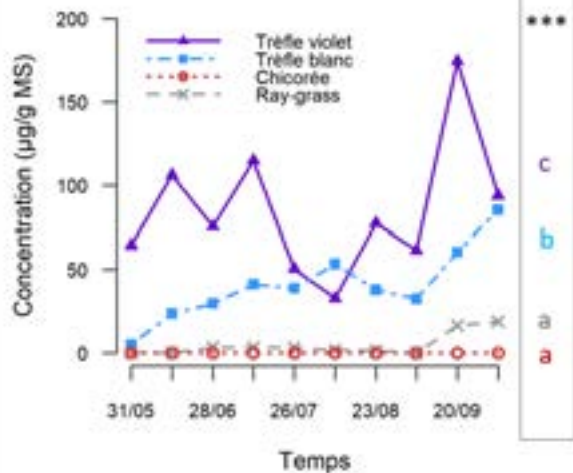
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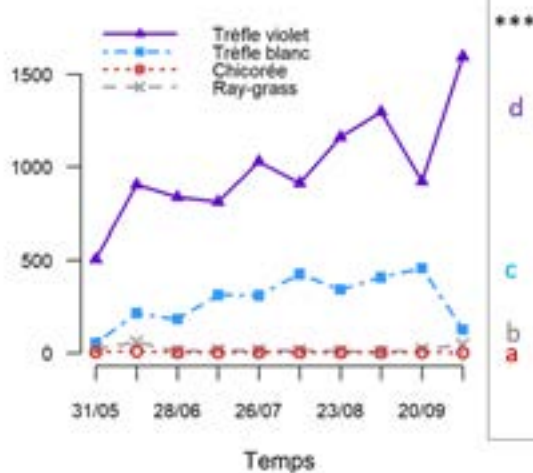
## Résultats



### Daidzéine



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[www.cra.wallonie.be](http://www.cra.wallonie.be)

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26

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## “quantitative-plant.org”: an online database of plant and crop models

*Saint Cast Clément*<sup>\*1</sup>, *Lobet Guillaume*<sup>1,2</sup>, *Couvreur Valentin*<sup>1</sup>, *Tardieu François*<sup>3</sup>, *Draye Xavier*<sup>1</sup>

<sup>1</sup> Earth and Life Institute, Université catholique de Louvain, 1348, Louvain-la-Neuve, Belgium

<sup>2</sup> Agrosphere, IBG3, Forschungszentrum Jülich, 52428, Jülich, Germany

<sup>3</sup> Université de Montpellier, INRA, MontpellierSupAgro, UMR LEPSE, 34060, Montpellier, France

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With the increasing number of phenotyping platforms and affordable image acquisition, images have become a commonly used data type in plant sciences. In the future, information extracted from phenotyping data will be used increasingly as parameters or variables of mathematical models, thereby broadening the scope of information extracted from phenomics data (Muller and Martre, 2019). Unfortunately, it is currently challenging for phenomics researchers to become aware of the diversity of models and their applications.

We present a manually curated database referencing plant and crop simulation models. The database is accessible online in a website, [quantitative-plant.org](https://quantitative-plant.org), which presents each model concisely in a consistent framework. Models are described by their general characteristics (e.g. programming language, plant part studied...) and uses (e.g. species studied...). In this way, [quantitative-plant.org](https://quantitative-plant.org) provides an overview of available plant image analysis software and models enabling users to identify available solutions for their project. We hope that this website will engage the interest of the phenomics community in model-assisted phenotyping.

### References

Muller B, Martre P (2019) Plant and crop simulation models: powerful tools to link physiology, genetics, and phenomics. *Journal of Experimental Botany* 70:2339–2344. doi: 10.1093/jxb/erz175

# GRANAR: the Generator of Root ANAtomy in R.

Adrien Heymans<sup>1</sup>, Valentin Couvreur<sup>1</sup>, Therese LaRue<sup>2</sup>, Ana Paez-Garcia<sup>3</sup>, Guillaume Lobet<sup>1,4</sup>

<sup>1</sup> Earth and Life Institute, UCLouvain, Louvain-la-Neuve, Belgium.

<sup>2</sup> Department of Biology, Stanford University, Stanford, United States.

<sup>3</sup> Noble Research Institute, LLC, Ardmore, Oklahoma, USA.

<sup>4</sup>. Agrosphere IBG3, FZJ, Jülich, Germany.

Root hydraulic conductivity is a limiting factor along the water pathways between the soil and the leaf, and root radial conductivity is itself defined by cell-scale hydraulic properties and anatomical features. However, quantifying the influence of anatomical features on the radial conductivity remains challenging due to complex time-consuming experimental procedures. We used the Generator of Root ANAtomy in R (GRANAR <http://granar.github.io>) to analyse maize (*Zea mays*) anatomical and create virtual anatomies from experimental observation. The generated anatomies were then used to estimate the corresponding radial conductivities and axial conductance with the hydraulic model MECHA [1] (Model of Explicit Cross-section Hydraulic Architecture). It enables the quantification of the effect of anatomical features on root hydraulic conductivities.

**[1] Couvreur V, Faget M, Lobet G, Javaux M, Chaumont F, Draye X (2018) Going with the Flow: Multiscale Insights into the Composite Nature of Water Transport in Roots. *Plant Physiol* **178**: 1689–1703**

## **Added value of plant height and multi-temporal modelling to improve the estimation of winter wheat, *Triticum aestivum* L., above-ground traits based on proxy-sensing RGB cameras.**

Sébastien Dandrifosse<sup>1\*</sup>, Alexis Carlier<sup>1</sup>, Esther Bustillo Vazquez<sup>1</sup>, Arnaud Bouvry<sup>1</sup>, Vincent Leemans<sup>1</sup>, Benjamin Dumont<sup>2</sup>, Benoît Mercatoris<sup>1</sup>

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Consumer-grade RGB cameras offer a low cost way to monitor crops but the descriptors extracted from RGB images (cover ratio, leaf color and texture) are not sufficient to fully describe the growth status of the crop. For winter wheat, *Triticum aestivum* L., many leaves and tillers are not visible in the images.

This research aims at investigating two approaches to improve dry biomass, Leaf Area Index (LAI) and plant nitrogen estimations : (i) take advantage of a second RGB camera to obtain leaf heights through stereovision (ii) exploit reference measurements and estimations of data acquired earlier in the season to enrich the models (Michez et al., 2018).

Reference measurements consisted of LAI, above-ground dry matter (leaves and tillers separated) and nitrogen at main wheat growing stages, i.e. BBCH 20, 32 and 39. Images have been acquired in field trials (Gembloux, Belgium) thanks to two RGB cameras positioned in nadir position (1 m above the crop). Extracted traits were canopy cover, vegetation indices and statistical descriptors of leaf heights. Simple, multiple and partial least squares regressions were tested.

Considering the best models, height information improved estimation of total dry matter with RMSE decreasing from 1.31 to 0.71 t/ha, RMSE of leaf dry matter from 0.34 to 0.28 t/ha, RMSE of total nitrogen from 23.37 to 18.44 kg/ha and RMSE of LAI from 0.63 to 0.56 m<sup>2</sup>/m<sup>2</sup>. Adding earlier estimations and reference measurements helped to improve models for given dates.

To conclude, this study highlights the importance of height as a predictor but it is assumed that proposed models might not be robust among wheat varieties for which further trials are necessary. A methodology to develop robust predictors is being developed.

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## **FUNDING**

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## PhenWheat: A field phenotyping platform for wheat experiments trials.

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Agriculture is facing new challenges for food security and sustainable development. Breeding is one of the keys to gain productivity using less inputs. It relies on both genotyping and phenotyping to identify some useful genes or traits as well as characterize and evaluate crops responses in various environments. Classical technics of phenotyping relies on human observations, which might be very laborious and often destructive. This still remains a bottleneck for crop improvement [1].

Advanced sensing technologies, computer science and robotics bring new accurate and non-invasive tools for high-throughput phenotyping (HTP). Despite those technological improvements, field-based HTP face many challenges due to the outdoor conditions encounter (wind, natural sunlight).

PhenWheat project aims at developing a field-based mobile phenotyping platform to characterize the growth dynamics of winter wheat varieties under different biotic and abiotic stress. The motorized platform will carry a stereoscopic vision system made from two RGB cameras, a multi-spectral camera, an incident light spectrometer and a thermal camera. RTK GPS will be implemented to follow micro plots throughout the season. Advanced image analysis and machine learning techniques will allow to process the data and to extract traits of interests (nitrogen and water stress, disease scoring, biomass, Leaf Area Index, plant height, ears density and plant nitrogen content).

The 2019 growing season has allowed to improve the automatization system and the acquisition method according to the outdoor working space, especially illumination conditions. The contribution will focus on the preliminary results regarding above mentioned traits.

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## Funding

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Page 24 of 207

## **Temporal assessment of photosynthetic capacity of winter wheat crops through reflectance spectrum and gases exchanges measurements**

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Imaging spectroscopy is increasingly employed to characterise the plant growth dynamics and to estimate photosynthesis and vegetation productivity of agrosystems [1, 2]. The analysis of the raw data provides information on a wide range of plant traits, such as nutrients and water content, photosynthetic capacity and biomass [5, 7, 9, 10]. Aboveground dry biomass estimations are often inferred from spectral indices, such as the Normalized Difference Vegetation Index (NDVI), normalized difference between reflectance of vegetation in the near infrared and the red regions [2, 4, 9, 10]. This index assesses the greenness of vegetation, i.e. the amount of chlorophyll content, indicating the potential photosynthesis. However, it does not allow a proper estimation of the actual photosynthesis which depends on the radiation use efficiency (RUE) dynamics of the vegetation [2, 4]. Under specific environmental conditions, changes in the reflectance spectrum can be measured to estimate the RUE of individuals. For instance, the Photochemical Reflectance Index (PRI), which is the normalized difference between reflectance at one stable (e.g. 570 nm) and one variable (531 nm) wavelengths, is related to one of the dissipation ways of excessive radiation, thereby not used for photosynthesis, by plants [4, 11]. Statistical models based on the whole leaf spectrum could also be considered to detect changes in RUE and assess the photosynthetic capacity at specific environmental conditions [2]. The advantages of spectral imagery in the estimation and prediction of photosynthetic capacity are the gain of spatial information and of sampling time.

Photosynthetic capacity can also be quantified with gases exchanges analyser tools (mainly CO<sub>2</sub>), which enable characterisation of several current photosynthetic rates [3, 6, 8]. Additionally, net primary production (biomass net carbon accumulation) can also be measured with destructive sampling and weighting of plants' material. Those measurements provide information on the actual radiation use efficiency and on physiological aspects of photosynthesis. They are suggested as validation and calibration of the information gained from the imaging spectroscopy methods.

In this context, this study will aim at evaluating the ability of imaging spectroscopy to assess and predict the dynamism of photosynthetic capacity across time of winter wheat (*Triticum aestivum* L.) in fields located in the Hesbaye area (Gembloux, Belgium), under contrasted agronomic management strategies. Data from spectral imagery at a proximate distance and from gases exchanges measurements at the leaf and individual scales will be collected seasonally to establish and predict their relationships with photosynthetic capacity. These predictions will be compared with destructive samplings and nutrient content analysis.

The designed method along with the accuracy of the expected results are discussed.

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## Identification of constraints affecting the coffee productivity and quality in Burundi

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The cultivation of coffee was introduced in Burundi in the early 1920s. It is currently cultivated in almost the whole country and represents 10% of the cultivated land. The favorable climate for coffee growing and the bourbon varieties which give high quality coffee are the great assets that the country has to develop this crop. However, the production and quality of coffee in Burundi is declining.

In order for the coffee sector to regain its competitiveness, a research project has been initiated on the agronomy of coffee in order to identify the agronomic and environmental constraints that affect the productivity and quality of coffee in Burundi. Data collection was carried out through a diagnostic survey to better understand the explanatory parameters of yield and quality. One hundred and ninety eight (198) coffee farms in the three main coffee production areas were targeted. The data collected were related to the description of the plot, plant vegetative parameters, yield parameters, soil fertility and the nutritional status of the coffee tree (soil and leaf sampling followed by chemical analyzes) as well as those in relationship with the sensory quality of the coffee.

Observed yields vary from 0.227 kg to 7.856 kg / coffee plant. Preliminary results revealed the effects of certain environmental factors on both yield and coffee quality. The exposure of the plots shows a highly significant difference ( $P < 0.001$ ) on the yield, with plots oriented towards the North and North-East providing a higher yield compared to those found on slopes oriented towards the West and North-West. The altitude of around 1,700 m was the most favorable for yield. A planting density of around 2000 to 3000 plants per hectare and clay loam soils were associated with the best yields.

Regarding coffee quality, 74% of farms produced merchant coffee classified as "FW15 +" with 56% classified "FWAA". It was also noted that from 1700m up to 1900m altitude, the acidity of the liquor has a very good to excellent score compared to plots located at a lower altitude, with the presence of citric and malic acidity and the limited presence of quinic acidity. For this same altitude, the flavor and the body were better. This is also valid for the physical quality of the coffee beans because from 1700 m to 1900 m the green coffee beans become heavier and heavier. Data related to the chemical quality of the soil are being analyzed in order to be able to draw further conclusions on their influences on the yield and quality of coffee in Burundi.

## **First steps towards automated early red mite detection in poultry using camera technology**

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The poultry red mite (PRM) is a major threat to the egg production industry worldwide. The prevalence of PRM is extremely high and increasing, causing big economic losses, animal health and welfare issues and public health issues. Routine monitoring of PRM forms an important aspect of Integrated Pest Management. It plays a key role in making pest control decisions and determining the efficacy of different treatments. Current PRM monitoring in hen farms commonly involves counting the number of mites that accumulate in passive mite traps. This method is far from optimal in three aspects: it is time consuming, the concentration of PRM populations can vary strongly within the henhouse, and the method focuses on the presence of the mites rather than on the welfare of the hens. With or without routine monitoring practices, farmers often decide to treat PRM infestations when the mites are visible in the henhouse. However, when the mites are visible, the infestation is already in an advanced stage.

The aim of this research project is to develop an innovative, automated monitoring system to replace current monitoring methods. Mite infestations make the hens become restless and agitated both during the day and night. This altered behavior can be captured by cameras resulting in an indication on the degree of mite infestation in the henhouse depending on the state of restlessness and agitation. This hen-based monitoring technique will serve as an early warning system to alert farmers that (extra) anti-PRM treatments are needed.

In this experiment, 32 laying hens were kept in 3 separate enriched cages. While the hens in cage 1 were kept as control group, the hens in cage 2 and 3 were infested with a small number of PRM. Their behavior and activity was monitored using both night vision cameras and depth cameras. Early results show a shift in behavior and an increase in nighttime activity with growing PRM population levels.

# Recursive feature elimination to improve the robustness of model predicting dairy cow bodyweight from lactation stage, parity, milk yield, season and milk mid-infrared spectra

Tedde A., Froidmont E., Dufrasne I., Wang Z., Gengler N., Dehareng F., Consortium G.P.L.U.S.E., Grelet C., Soyeurt H.

From the dairy farmer's perspective, knowing the body weight (BW) of a cow at a specific moment or measuring its evolution through time is of huge interest. Various methods exist to assess BW, but either they are too expensive and need costly and time-consuming maintenance to be in production at a large scale, or their application occurs only once during the animal lifetime. A literature model to predict BW [1], including animal-unspecific (days in milk, parity, season) and specific data (milk yield and milk mid-infrared spectra), employs 283 predictors. This study claims that the use of a smaller subset of these predictors is enough to obtain at least equivalent prediction accuracy (i.e., cross-validation root mean square error, RMSEcv). Minimizing the number of predictors implies a less complex model that reduces the risk of overfitting. So, a recursive feature elimination, applying linear regressions, partial least square regressions (PLSR), and random forest, was performed on 1,802 records, to select the most important variables, which were then included in PLSR to predict BW. The latter outputs a RMSEcv of  $43.43 \pm 1.91$  kg based on 109 predictors. The PLSR, including all the 283 predictors, had a RMSEcv of  $44.75 \pm 1.51$  kg. Consequently, we can conclude that the PLSR calibrated with fewer variables produces similar prediction accuracy. The reduced model presents the advantage to be less complex and, therefore, more robust compared to the full model found in the literature.

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## Session 1 (BV)

# Food technology, safety & health

11h15 -12h15

**Chairman: Bram Van den Bergh – KULeuven**

Influence of triacylglycerol composition on the baking performance of palm-based puff pastry margarines  
Roxane Detry (ULiège)

Can coagulase-negative staphylococci play a role in the safety of fermented meats? Assessment of antibacterial activity and biogenic amine formation  
David Van der Veken (VUB)

Hybrid fractionation process for Faba bean protein extraction: effect of combining dry and wet extraction steps on anti-nutritional factors content  
Lionel Dumoulin (ULiège)

2-Aminoimidazoles as a potent inhibitor of brewery biofilms  
Lene Jacobs (KULeuven)

Flash presentations

## Development of a new highly specific method for the identification and quantification of the main allergen Lup an 1 of narrow-leaved lupin (*Lupinus angustifolius* L.) seeds in natural and processed foods

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Lupin is a legume increasingly demanded for consumption due to the large number of health benefits (functional food), particularly narrow-leaved lupin or NLL (*Lupinus angustifolius* L.).

This rapid introduction and growing market demand for new foodstuffs based on components of lupine seed, the number of people allergic to lupine is also growing [1,2,3]. Thus, the aim of this study was to develop a new method to be used for the detection, identification and quantification of the main lupin allergen (Lup an 1) in both processed and unprocessed foods.

This method offers absolute specificity in the identification of beta-conglutin proteins, which are the main allergens of the lupin seeds. This technology can also detect cross contamination in foods that typically do not contain them [4,5].

The specificity and novelty of the method is based on 1) the development of a highly specific antibody by combining bioinformatics and experimental methodologies, 2) obtaining this allergen (Lup an 1 / Lup a 1) purified from recombinant sources, 3) the use of these two in obtaining standard quantification curves, and 4) in the method of protein extraction specially adapted for food, thereby avoiding obtaining "false positive" detections due to cross reactions with other proteins of nearby species such as peanut, and even with non-allergenic proteins; or "false negatives" due to the absence of these allergens due to the inappropriate method used for the extraction of protein from food [6].

This new technological development will contribute to a more effective management of food allergens by the food industry, regulatory agencies and bio-sanitary professionals, thus helping to protect the health of consumers.

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## Seed $\beta$ -conglutin proteins from narrow-leaved lupin (*Lupinus angustifolius* L.) as functional foods and their role in cancer prevention

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*Lupinus angustifolius* (narrow-leaved lupin, NLL) seeds are a valuable source of proteins for human consumption with multiple nutritional and nutraceutical properties [1]. Among these proteins, particular research attention has been focused to those from the vicilin or beta-conglutin family, which are the most abundant proteins in lupin seeds [2].

Recently, it has been demonstrated the potential anti-diabetic [3], insulin sensitivity improvement [4], antioxidant and anti-inflammatory activities of particular beta-conglutin proteins ( $\beta 1$ ,  $\beta 3$  and  $\beta 6$ ) [3,4,5]. These properties could be attributed to the ability of these conglutins to interact with insulin [3] and their particular structural characteristics [6,7].

Up until now, there is no information concerning how NLL seed proteins affect cancer cell at molecular level. The aim of this study was to evaluate the effects of NLL  $\beta$ -conglutin proteins on human breast cancer cells by clonogenic and viability assays.

These studies allowed determining the optimal  $\beta$ -conglutin proteins concentration ranges of each isoform to know the percentage of breast cancer cells surviving in comparison to control healthy cells, and whether there was sensitization of these cancer cells after treatment with x-ray radiation at different doses (1 Gy to 8 Gy) alone and in combination with  $\beta$ -conglutin proteins treatment.

NLL  $\beta$ -conglutin proteins may be an agent with potential uses in combinatorial therapies helping to fight human breast cancer.

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**Variabilities of *Tetrapleura tetraptera* (Schumach & Thonn.) taub. and *Aframomum citratum* (C. Pereira) K.Schum from three Agro-ecologic zones in Cameroon and effect of extraction methods on the phenolic compounds recovery**

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The fruits of *Tetrapleura tetraptera* and *Aframomum citratum* were identified<sup>1</sup> among the most commonly used and available spices in Cameroonian cities. They are mainly collected in the bimodal forest (Zone V), unimodal forest (Zone IV) and the highlands (Zone III)<sup>2</sup> zones. They are part of the traditional pharmacopeia in Central and West African countries<sup>3-6</sup>.

The aim of this study was to characterize fruit samples harvested on the three agro-ecological zones (AEZ) and to examine few traditional extraction methods for the phenolic compounds recovery in a domestic food consumption situation. *Tetrapleura tetraptera* fruits were harvested in twelve villages spread over three AEZ (Zones V, IV, III) and *Aframomum citratum* fruits were selected in nine villages spread over two AEZ (Zones V, IV). Various analysis (dry matter, ash, total fat, crude proteins, total sugars, crude fibre contents; energy, water activity and pH; Fe, Ca, Mg and K contents; total polyphenols and flavonoids contents) using standard analytical, colorimetric, spectroscopy and spectrophotometric procedures were used. For both extraction methods investigated (Maceration, infusion, decoction), an extraction ratio of 1/5 was used. Statistical analysis and Principal Component Analysis (PCA) were performed.

The results showed that concerning *Tetrapleura tetraptera* samples, crude fibre content is significantly ( $p < 0.05$ ) high on samples from Zones IV and III while samples from Zone V possess more sugars, calories, minerals and phenolic compounds. About *Aframomum citratum*, samples from Zone IV are the most sweet and the ones from Zone V contain more protein, fibre, phenolic and minerals (especially samples from the East region) contents. For both species, decoction was the most efficiency extraction method. The result gives a graphic representation of the studied characteristics which can serve as a decision-making tool (for sampling) to guide the various stakeholders involved in the valuation and exploitation of these fruits.

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## Palm oil dry fractionation with palm stearin blending

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Palm oil is the most consumed oil worldwide. Its composition confers technological properties that no other vegetable oil can attain. It is also the most fractionated oil with a huge potential in multi-step dry fractionation. Different routes are clearly established leading to specialty products like low IV super stearin (solid route), high IV top olein (liquid route) and hard palm mid fraction (ingredient for CBE).

This work focused on the liquid route, more particularly on the first step: production of olein (IV 56) and stearin from palm oil. The initial objective was to find a way to improve the olein yield thanks to an optimization of the palm oil composition with stearin blending. To this end, palm oil was blended with increasing amounts of palm stearin to obtain matrices with variable tri-saturated triglyceride contents; five blends were investigated. Dry fractionation was carried out on pilot scale using a Tirtiaux crystallizer coupled to a high-pressure membrane press filter.

After optimization of the cooling curves, on-going crystallization was monitored by sampling using optical microscopy and powder X-Ray diffraction. Crystallization kinetics were derived from solid fat content of the slurry by p-NMR, iodine values and DSC cloud points of the olein after vacuum filtration. Press filtrations were then performed at identical temperature after the same crystallization time. Fractions quality was evaluated based on iodine value, DSC cloud point and triglyceride composition by HPLC.

It was shown that one specific matrix could release more olein after press filtration in identical conditions. This olein was characterized by slightly higher iodine value and surprisingly higher DSC cloud point. This matrix was also the fastest to achieve a steady state during the crystallization kinetics.

All the analytical tools used helped in understanding and trying to elucidate this unexpected behavior.

## Effect of maturation temperature and starter cultures on the rate of cream acidification

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### Abstract

Butter is a dairy product rich in fat, but it also contains an aqueous phase, a good medium for microorganisms [1]. The growth of microorganisms can be prevented by the application of certain treatments like reducing pH.

In raw milk butter production process, cream fermentation is a fundamental step to reduce pH [2]. In a previous study, it was found that this operation presented variations among producers in terms of the conditions of maturation applied [3].

The aim of this work was to study cream pH evolution in relation to the conditions of maturation applied, especially temperature and addition of starter cultures. Four comparative trials of maturation were conducted taking into account these two factors. Cream pH and temperature measurements were recorded every 30 minutes for 5 days in a row.

It was found that cream acidification did not occur in the fridge, and that cream pH decreased significantly at 14°C. At this temperature, the rate of acidification was also faster in the presence of starter cultures.

This study has allowed to highlight the effect of starter cultures as well as the temperature of maturation on the rate of acidification. It is thus important to control the maturation conditions. The use of starter cultures can enhance the acidification of the cream. Nevertheless, even without starters, a sufficient maturation period at a proper temperature can also lead to a good fermentation.

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## Effects of processing and storage conditions on the stability of sweet potato (*Ipomoea batatas* L.) leaf flavonoids

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China is the leading sweet potato (*Ipomoea batatas* L.) producer in the world, the annual yield of 2017 was 72 million tons, accounting for 64 % of the total yield (FAOSTAT, 2017). After harvesting the roots, there is almost equal amount of sweet potato leaves (SPL) waiting to be discarded. SPL is rich in polyphenols ( Sun et al., ) and flavonoids is the main branch of which has the typical C6-C3-C6 molecular structure that possess high antioxidant activity, could slow down chronic diseases, such as rheumatism, cardiovascular diseases and cancers (Chen et al., 2019).

The stability of flavonoids during processing and storage is critical to factories and consumers, in order to attain the desired nutritional and functional effects, heat treatment and high hydrostatic pressure (HHP) treatment were employed to attain processing and bactericidal effect (Li et al., 2017).

The overall stability of SPLF during processing and storage was evaluated and the results showed that heat treatment at 75 °C for 90 min or HHP treatment at 600 MPa for 30 min didn't cause significant effect on SPLF. When the temperature reached up to 100 °C for 60 min even 90 min leading to a decrease in antioxidant activity by 20 % and 25 % respectively, while pH 7.0 and 8.0 significantly decreased amount of SPLF by approximately 75 %, decreased antioxidant activity by about 30 % and 47 % separately. Light treated samples recorded a decrease in SPLF by 52 % and antioxidant activity by 24 %. No significant effect on SPLF was observed for samples stored at -18 °C, 4 °C or room temperature (RT). The retention of flavonoids and antioxidant activity was 45.9±3.6 % and 56.2±2.6 %, individually in SPLF after simulated digestion.

## **Influence of triacylglycerol composition on the baking performance of palm-based puff pastry margarines**

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In the past, partially hydrogenated fats rich in *trans*-fats have been used widely in lamination margarines (roll-in fats) because they provide superior plasticity, essential for a perfect layering and pastry lift. However, the adverse health effect of *trans* fatty acids has urged industry to move away from partially hydrogenated fats. Due to its versatility, palm oil is usually used to design *trans* free functional fats such as puff pastry margarine. *Trans* fats crystallize quite easily in the right form and are quite stable. However, palm oil and derivatives have to be carefully selected and balanced to avoid undesirable crystal transformations that could be detrimental to the end product quality. In this study, the evolution of physicochemical properties and baking performance of 3 *trans*-free palm-based puff pastry margarines was investigated up to 6 months storage. The 3 products possessed similar fatty acid but different triacylglycerol compositions. Palm oil, palm stearin, interesterified palm oil and palm mid fraction were used to obtain different proportions of the main triacylglycerols found in palm products (PPP, POP, PPO, POO and OPO). Their behavior was compared to a palm-based margarine containing *trans* fatty acids. The 4 margarines were produced at pilot scale using the same processing parameters, based on the same emulsion formulation -only differing in fat composition.

The reference margarine was stable and possessed satisfying baking properties over the whole storage period. On the other hand, the *trans* free margarines showed distinct behaviors, evolving differently depending on their triacylglycerol composition. Results showed that fat composition influences short term as well as long term performance of the products. It is therefore necessary to adequately balance the triacylglycerol

composition in order to avoid or delay polymorphic transformations that can dramatically affect the quality of palm-based puff pastry margarine.

## **Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc × (Landrac × Yorkshire) pigs by volatiles profiling and chemometrics analysis**

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To characterize and differentiate boiled pork from three different breeds of pig (Tibetan, Sanmenxia and Duroc × (Landrace × Yorkshire)), the volatile compounds in each were analysed by gas chromatography-olfactometry-mass spectrometry (GC-MS/O) and electronic nose (E-nose) combined with chemometrics analysis. In total, 61 volatile compounds were identified, among which 25 compounds were selected as odour-active compounds in boiled pork. Moreover, seven odour-active compounds (hexanal, nonanal, 1-octen-3-ol, dimethyl disulphide, heptanal, 2-pentylfuran and 2-ethylfuran) were the main contributors to the integral flavour of boiled pork due to their higher odour activity values (OAVs) ranging from 17.3-524.2. The odour-active compounds were examined by principal component analysis (PCA), agglomerative hierarchical clustering (AHC) and partial least squares-discriminant analysis (PLS-DA). The results showed that boiled pork from the three pig breeds could be clearly distinguished, and twelve odour-active compounds, including (E,E)-2,4-decadienal, ethyl hexanoate, dimethyl disulphide, hexanal, 2-acetylthiazole, (E)-2-nonenal, 1-octen-3-ol, (E,E)-2,4-nonadienal, heptanal, (E)-2-octen-1-ol, styrene and (E)-2-octenal, were determined as potential flavour markers for discrimination. This study indicated that GC-MS/O and E-nose with chemometrics analysis are feasible methods to characterize and discriminate boiled pork from three pig breeds.

## Hybrid fractionation process for Faba bean protein extraction: effect of combining dry and wet extraction steps on anti-nutritional factors content

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Faba bean, like any other legume, has seen its interest by the public and the agro-food industries growing up these past years. With a protein content as high as 30 % of the seed dry weight (DW), Faba bean is a serious candidate to replace meat products and ingredients in our everyday life. More than the agronomical advantages of cultivating Faba beans, those proteins have a high nutritional value and functionalities suitable for food products integration.

Industrially, legume proteins are extracted by dry or wet processes, each having its pro's and con's. To combine both processes type advantages, a hybrid one was developed at laboratory and pilot scale. After a dry fractionation producing a protein-rich and a starch-rich flour, the latter is subjected to a wet protein extraction, producing another protein-rich flour and a protein-free starch-rich flour.

With a high protein recovery in the 2 fractions of interest (around 90 and 65 % at laboratory and pilot scale, respectively) and high protein contents (around 67 and 90% w/w DW at laboratory scale and 58 and 61 % w/w DW at pilot scale), the developed processes show better results than generally presented in the literature.

However, isolating Faba proteins has the disadvantage to concentrate anti-nutritional factors (ANF), such as phytic acid (lowering the bioassimilation of Iron, Zinc and Calcium), trypsin inhibitor (inhibiting the digestion of proteins) and (con)vicine, (exclusively found in Faba bean, lethal for people with G6PD deficiency) in the fractions of interest. The amount of each ANF found in the produced fractions is dependent on the process parameters as well as the Faba varieties.

To validate the developed fractionation process for food purposes, keeping track of the ANF contents and their possible impacts on human health is essential. More than just quantifying the ANF contents, they must be compared to regulations and/or recommendations.

## Can fungal volatile organic compounds be used to develop aflatoxin-specific sensors?

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### Abstract

Foodstuff (corn, wheat, rice, etc.) can be contaminated by several filamentous fungal species in pre or post-harvest conditions. Some of these, such as *Aspergillus*, *Fusarium* and *Penicillium* produce secondary metabolites, highly toxic at low concentrations to all vertebrates including humans: they can cause severe illnesses upon chronic exposure and can even lead to death after acute exposure. These non-volatile molecules are named mycotoxins and current methods to detect them, involving the use of ELISA tests or HPLC, are quite time consuming and expensive. At present there is no rapid test that does not require extensive sample preparation to detect the presence of mycotoxin directly in a production line (e.g. grain storage companies). Therefore, the aim of this work is to identify volatile organic compounds (VOCs) markers, specific of mycotoxins' production in foodstuff.

Using the SPME technique, we have characterized and compared the VOCs produced in vitro by non-aflatoxigenic (not producing aflatoxins) and aflatoxigenic strains of *Aspergillus flavus* (producing aflatoxins B1, B2 and G2, three types of mycotoxins). Preliminary analyses have shown similarities and differences between the two strains. Both of them emit VOCs as 1-octen-3-ol, 3-methylbutan-1-ol, octan-3-one, 2-methylbutanal, 3-methylbutanal, known in the literature to be specific of fungi. In particular, we have identified several strain-specific terpenes that are of interest for the development of the future molecular foot-print sensor.

The next step is to study the VOCs produced in in vivo conditions, when the fungi are growing on stored cereals; and the correlation between specific VOCs and mycotoxin production.

## Characterization of the microbial diversity of Belgian artisanal cheese using metagenetics

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More than 450 artisanal cheeses have been identified in Belgium during a survey, including some typical products, like Maquée, Herve or Abbaye. Microbial communities vary between types of cheeses. Cheese microflora can originate from raw milk, production and ripening environments, or intentionally added starters<sup>1</sup>. Moreover, cheese cores and crusts generally represent two distinct ecosystems. Internally, anaerobic lactic acid bacteria are generally found, while an increased microbial diversity is observed on cheese surface, where oxygenation allows the growth of bacteria, yeasts and molds<sup>1</sup>. Microbial communities of cheeses are thus extremely complex. The main goal of this study was to characterize the bacterial diversity of Belgian artisanal cheeses. Twenty-six artisanal cheeses, including unripened, soft and semi-soft cheeses, were studied in triplicate. Samples were collected in 26 farms directly after production or after ripening, for unripened and ripened cheeses, respectively. After DNA extraction and amplification of regions V1-V3 of the gene coding for bacterial 16S rRNA, DNA was sequenced using Illumina MiSeq technology. Obtained sequences were grouped into operational taxonomic units (OTUs) by phylotype at the species level, using Mothur<sup>2</sup>. Conserving 10,000 sequences for each sample, 3,201 OTUs have been identified. These OTUs belong to five major phyla. At a deeper taxonomic level, only 16 genera represent more than 0.1 % of the sequences in at least one type of cheese.  $\alpha$ - and  $\beta$ -diversity were also studied and compared between types of cheese.

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**Fermentation of rare sugar kojibiose by the oral bacteria is slow yet bacteria specific**

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**Abstract**

Sucrose is a common ingredient in many foods owing to its desirable technological and sensorial functions (1); however, its ease of fermentation by the diverse oral microbiota is associated with dental caries that affects over 2.5 billion people globally (2, 3). In this study, we have investigated the cariogenic properties of 1% rare sugars; kojibiose and trehalose as potential sucrose alternatives. Sugar incubations were conducted anaerobically for 24 and 48 h in a carbon-limited medium with *in vivo* derived complex microbial communities of saliva from 11 donors and with selected oral bacteria as either single strains (19) or their synthetic communities. Based on the levels of pH and organic acids, kojibiose resisted rapid fermentation by the complex salivary microbiota from all the donors except one. As for pure strains, not all single strains except *A. viscosus* at 48 h were able to metabolize kojibiose highlighting the resistance and strain specificity in kojibiose utilization. A synergistic metabolic interaction was, however, revealed for kojibiose incubations with communities comprising either lactobacilli or *A. viscosus* at 48 h. On the contrary, sucrose and trehalose were rapidly fermented by most of the single strains, all the synthetic communities and salivary bacteria thereby producing comparable high levels of organic acids that coincided with the drop in pH below the critical level 5.5. Taken together, our results have demonstrated that kojibiose is a low cariogenic disaccharide hence a potential sucrose alternative as opposed to trehalose that exhibited comparable cariogenic properties as sucrose.

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## COMPREHENSIVE MULTIDIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH MS AND FID (GC×GC-MS/FID) FOR MOSH&MOAH DETERMINATION IN FOOD

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The presence of mineral oil hydrocarbons (MOH) in food was reported for the first time by Biedermann and co-workers<sup>1</sup> in irradiate hazelnuts in 1989. However, such a contamination problem was only brought at the attention of the public opinion 20 years later when the same authors related the presence of the high amount of MOH find in dry food to the use of recycled fibers for food contact material<sup>2</sup>. The analysis of such a contaminant in food is a challenging task, due to the high affinity of MOH with the lipid fraction and the co-extraction of many lipid interferents from some samples, as palm oil. MOH is compose by two main fraction, namely saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH), with different analytical challenges and toxicological concern.

The on-line LC-GC/FID method was proposed by the European Food Safety Authority (EFSA) as the most effective method for the routine analysis of both MOSH and MOAH, but the presence of false positive and the doubts on the nature of the chromatographic hump originated with this analysis remain, due also to the lack of a proper confirmatory method as required by the EU Decision 2002/657.

The goal of the present study is to optimize a GC×GC-MS/FID after the LC separation for the simultaneous quantification and confirmation of the results. A careful optimization is necessary to proper elute up to C50 (as requested by the European Joint Research Center (JRC) Guideline<sup>4</sup> on the minimal requirements of the method to be used) without losing the inherent advantage of structured chromatograms obtained when using GC×GC.

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## Characterization of the chemical communication of the poultry red mite, *Dermanyssus gallinae* (Acari: Dermanyssidae) and its potential use in biological control

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*Dermanyssus gallinae* (De Geer, 1778), commonly named poultry red mite (PRM), is considered as the most harmful ectoparasite in poultry farms in Europe. This species feeds from blood of laying hens but spend most of its time hidden in cracks and crevices around hen's nests. To control PRM populations in poultry houses, chemical pesticides are currently used with growing concern on harmful residues found in eggs and hens and mite resistance against several compounds. Other alternatives are now explored to avoid the use of synthetic compounds such as vaccines, biological control, physical control or semiochemical control base on PRM chemical ecology. Our study focuses on the identification of pheromones emitted by PRM such as sex pheromone. To that purpose, samplings of individuals were realized in poultry houses and then, a first volatile organic compounds (VOCs) sampling was realised by SPME (Solid Phase Micro-Extraction) coupled to GC-MS (Gaz Chromatography – Mass Spectrometry) analysis to identify VOCs realised by the crush of 300 female individuals. Results allowed to identify five compounds never found in other Acari species. However, one compound, 2-Propyl-1-pentanol, was already found in feces extracts of Bedbugs *Cimex hemipterus* (Fabricius, 1803).

These preliminary results allow to discuss potential changes of parameters for future studies. Other VOCs sampling technics are considered and future behavioral experiments are planned. Perspectives for an application of the attractive compounds in the field are evocated. They could be used in an "attract and kill" trap if coupled to a biological acaricide such as entomopathogenic fungi or silica dust.

## Evaluation of some quality parameters of crude shea butter produced in Burkina Faso

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Gross composition and physicochemical properties of shea butter (SB) widely vary depending on geographical origin, cultivars and extraction process. This study aims at characterizing the composition of crude SB collected in Burkina Faso, with a particular interest for the unsaponifiable. Results concerns five samples, all obtained by traditional extraction. The fatty acid (FA) profile (esterified and non-esterified) was analyzed by GC/FID, using an optimized method. Acidity, Iodine (IV) and Peroxide values (PV) were determined by titration.

As expected, the main esterified FA (mean values) were: C18:1 (cis-9) (42.07%); C18:0 (40.38%) and (C18:2) n6 (5.61%). The FFA content ranged from 2.27 to 4.17%; the IV from 60.60 to 63.51 and the PV from 6.23 to 9.31 meqO<sub>2</sub>/kg. The unsaponifiable content was found between 7.24 and 13.50%.

In this preliminary study, a great homogeneity was found among the samples. This next step about a large range of samples will be an investigation of some absorbent properties of unsaponifiables using HPLC, UV, IR and FT-Raman spectroscopy, in order to determine relationships between extraction processes

## Trace element and essential amino acids content in *Longissimus Lumborum* muscle from three sheep meats reared in outdoor conditions

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**Abstract:** Meat is a major source of nutrients for human nutrition, especially trace elements and many essential amino acids (EAA). Because of its high nutritional value, meat is considered as an essential food necessary for a balanced human diet. The study aimed to search the distinctive aspects of the nutritional quality of three sheep meat produced on Moroccan pastures, in terms of the trace element and the EAA. The results show that the breed and geographical area (GA) affect significantly the mineral and AA composition ( $p < 0.05$ ). Trace element content range between a minimum of 5.36 mg/100g of fresh meat for Ouled-Djellal (ODj) and a maximum of 5.7 mg/100g for Timahdite (Ti). Iron and zinc were the most abundant minerals, while the selenium (0.013-0.021) was the least abundant. Except for Fe, all identified trace elements were influenced by breed effect, while they are not affected by GA ( $p > 0.05$ ). Quantitative and qualitative analysis of the amino acid profile allows us to identify 17 amino acids, including 8 EAA. Leucine (3.31 - 4.8%) and lysine (3.69 – 4.17%) are the two most abundant EAA. Thus, the lamb meats analyzed show: (i) a richness in amino acids which varies between a minimum of 24.17% for Beni-Guil lamb meat sampled in the Aîn Beni-Mathar region (BGA) and a maximum of 27.20% for Ti; (ii) protein values with an essential amino acid number between 100.86 and 115.06 respectively for lamb meat BGA and Ti.

**Key words:** Sheep, Breed, Meat, Amino acids, Minerals

## Vacuum-Assisted HSSPME, An Innovative Sampling Approach, For Food Applications

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**Keywords:** Foods, Gas Chromatography Mass Spectrometry (GC-MS), Volatiles, Vacuum-assisted HSSPME

Vacuum assisted headspace solid-phase microextraction (Vac-HSSPME) has been proposed as a valid technique to increase the performance of conventional HSSPME.

Although the total amount extracted at the equilibrium does not change when reduced pressure conditions are applied, an improvement of extraction yield is observable at shorter extraction time and milder extraction temperature compared to conventional applications. This is particular beneficial for analytes with a low affinity for the headspace (semi-volatiles).

The use of Vac-HSSPME is of particular interest for the analysis of food volatiles, allowing to obtain a more comprehensive evaluation of the aroma profile, especially when heating the sample can easily cause artifacts formation.

The effect of reduced pressure conditions on a completely fatty sample, i.e. extra-virgin olive oil, has been investigated for the first time and new insight into the SPME mechanism have been obtained.

In the present work, the effects of extraction temperature and sampling time were investigated using both traditional one variable at a time approach and a two-variable central component design (CCD).

Moreover, Vac-HSSPME has been used to sampling the volatile compounds of raw fish at refrigerated temperature obtaining results comparable to regular SPME at room temperature

## Meat quality and salt concentration influence the bacterial species diversity and community dynamics during fermentation in meat models, potentially leading to quality concerns

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Acidification level and temperature modulate the beneficial consortia of lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS) during meat fermentation. Less is known about the impact of other factors, such as meat quality and salting [1, 2]. These could for instance affect *Enterobacterales* outgrowth, potentially indicative of poor fermentation practice [3]. Therefore, pork meat batters from either normal or borderline quality (dark-firm-dry, DFD) were compared at various salt concentrations (0-4 %) in fermentation models. Whilst *Lactobacillus sakei* governed the normal meat, *Lactobacillus curvatus* was more prominent in the DFD variant. CNS were favoured at rising salt concentrations without much effects on species diversity, consisting mostly of *Staphylococcus equorum*, *Staphylococcus saprophyticus*, and *Staphylococcus xylosus*. In DFD meat, *S. saprophyticus* was less manifest than in normal meat. *Enterobacterales* mainly emerged in DFD meat at low salt concentrations. The salt hurdle seemed to be insufficient to prevent *Enterobacterales* when acidification and initial pH were favourable for their growth. In conclusion, the results showed that difference in meat quality significantly influenced the bacterial community dynamics, while salt variation had less impact. This study highlighted concerns related to the outgrowth of *Enterobacterales*, especially in DFD meat fermentation with low salt concentration.

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## **The Flemish vaginal microbiome in pregnancy shows 5 distinct profiles in the first trimester**

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The microbiome field is subject to a growing interest of the female reproductive health, and the closely associated health of their children. The vaginal microbiome has already been suggested to influence vaginal disease prevention, successful fertilisation and healthy pregnancies. Vaginal dysbiosis is often characterised by a vaginal microbiome not dominated by *Lactobacillus* species. However, the exact link between lactobacilli and health is not yet well understood. As the worldwide incidence of preterm birth is more than 10%, strategies to reduce this number are in high demand. In this study, we aim to gain detailed insights in the Flemish vaginal microbiome composition and dynamics of 1000 women through three semesters of pregnancy and compare this with a non-pregnant healthy reference population. At the moment, we collected samples from 125 pregnant women at week 7-14 of gestation. The first results with 16S rRNA sequencing showed five distinct vaginal microbiome types where the women were mainly dominated by one of four *Lactobacillus* species: *L. crispatus* (26,4%), *L. iners* (32,0%), *L. gasseri* (12,0%) or *L. jensenii/fornicalis* (10,4%), which are all thought to be beneficial for vaginal health. However, the rest of the women had a higher microbial diversity (19,2%) not dominated by lactobacilli. Presence and relative abundances of the microbial communities are correlated with various metadata. We will follow-up with longitudinal sampling and special attention will be given to the group with a diverse microbial profile. This study aims to contribute to a better understanding of the composition and diversity of the maternal vaginal microbiome, its link to preterm birth risk and provide clues on novel potential maternal probiotics.

## IDENTIFICATION OF CASHEW APPLE VALORIZATION PATHWAYS BY PHYSICOCHEMICAL CHARACTERIZATION

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### ABSTRACT

To consolidate its position as the world leader in cashew production (with nut yields of 750,000 tonnes in 2018), Ivory Coast has to improve its productivity and strengthen its potential for local processing of this agro-resource. The cashew tree has two morphological parts that are the nut (real fruit) and the apple (false fruit). If, however, the cashew nut is of obvious interest, very little valorization is made of the apple although it could present interesting nutritional and nutraceutical properties with a biomass 10 times higher than that of the nuts.

This study aims to characterize the cashew apple in order to identify its characteristics, with a view to better valorization. For that purpose, two varieties of cashew apples from Ivory Coast, from two different production zones were selected and dried before physicochemical characterization.

The first part of the study on the collection, drying and grinding of apples showed that oven drying was more adequate and bagasse would be easier to process than whole apples. The second part dealing with the physicochemical characterization of dried apple powders, dried bagasses and cashew apple juice, showed that the juices contained more sugars (53.65%), less fat (0.74%) and a low protein content (1.43%). On the other hand, bagasses, which are richer in protein and fat, with 11.46% and 5.35% respectively. Red apples dried in an oven are less rich in sugars with a rate of 24.19% (in dry weight basis). In addition, red apples are richer in protein and drying in oven gives a higher protein

NSABS 2020

content. Indeed, the study showed the unequal distribution of constituents between these samples. Therefore, the study of the apple, for a better valorization would be more interesting with a juice extraction for the production of drinks and a by-product (bagasses) valorization as a source of protein. Thus, cashew apple powder could be used as an ingredient in order to offer foods enriched with functional molecules.

**Keywords:** cashew apple, physicochemical characterization valorization, food, ingredient.

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NSABS 2020

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## Effect of region of almond trees growing and Almond varieties on the quality of their almond oils

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Belgium supports Morocco in its development strategy for inclusive and solidarity farming. Eastern Morocco benefits from a major integrated project (PROFAO) for the sustainable development of the almond's sector. Thus, 6000 hectares of almond trees are planted and units for almonds shelling, storage and valorization of almond products are set up. This study concerns the evaluation of effects of the growing area and almond varieties on the quality of almond oils (AOs). Thus, mechanically extracted oils from 4 varieties (*Ferragnes (Fg)*, *Ferraduel (Fd)*, *Fournat (Ft)* and *Marcona (Mr)*) grown in 2 regions (Rislane and Tansherfi) were analyzed for acidity (IA), peroxide index (IP) and oxidative stability (SOI). This permit us to compare shelf stability of AOs to be use as edible oil or for cosmetic, because quality of oil greatly affects its shelf life. Results show low values for IA (0.3%; 0.47%; respectively for *Ft* in both regions; and for *Mr* in Tansherfi) and IP (1.75; 3.64 meq/O<sub>2</sub> respectively for *Mr* in Tansherfi and for the *Fd* in both zone). SOI values range between 20.76 and 27.8 hours for *Fg* in Rislane and *Fd* in Tansherfi. Analysis of variance shows significant influence (P=0.05%) of region and variety on IP and SOI of AOs, However, no significant effect on IA was observed. According to SOI values, AOs of *Fg* and *Fd* varieties grown in Tansherfi show best shelf stability than those produced in Rislane, while AOs of *Ft* and *Mr* are more stable when produced in Rislane. Finally, in both regions, low values for IA and IP for AOs of all varieties and high values of (SOI) especially for *Fd* and *Fg* varieties are important indicators for quality of AOs as an edible oil but also for its recommendation for cosmetics uses that generate high added value and improve farmers' income

**Keywords:** Almond Oil, Acidity, Peroxide index, Oxidative stability indices

## Development of a lab-on-a-disk platform for detection of parasite eggs in animal stool

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We recently developed (in collaboration with J&J) a lab-on-a-disk platform based on centrifugal flotation for fast identification and quantification of parasite eggs in stool<sup>1</sup>. The concept of our technique was related to the Cornell Wisconsin method. The transfer of eggs from centrifugation tube to microscope slide has been largely neglected in this process, leading to an error-prone procedure and weak sensitivity compared to other methods<sup>2</sup>. With our approach integrating centrifugation and visualization into a single platform improved sensitivity as the requirement for egg transfer is eliminated. We implemented essential new features to improve the PMMA-based diagnostic performance, such as *guided flotation*, *continuous size-based filtering* and *single shot imaging*. In the current contribution, results of experiments that we have performed using a new generation of devices performed in Uganda with stool samples from cattle infected with intestinal parasites are shown. 1g of solid stool was suspended (6-step procedure) in 0.5mL flotation solution (saturated NaCl). The sample was injected using a plastic syringe and eggs were successfully isolated and collected within the imaging zone at 5000RPM in 5min. The experiments showed that enhancing the flotation behavior of parasite eggs by employing microfluidics and centrifugation for their collection in a single imaging area provided clear advantages regarding digital data creation as well as easier identification and quantification.

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## Towards an optimal leaf area/ fruit mass ratio of *Vitis vinifera* cv. Chardonnay in Belgium for optimal consumer preference

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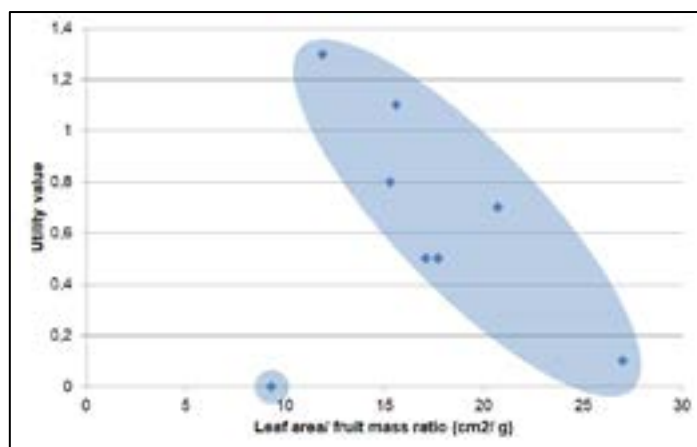
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In Belgian viticulture yields are held artificially low by bunch thinning. Leaf area and bunch number have to be regulated conjointly to obtain proper source-sink ratios. Photosynthesis, lateral shoot growth, amino acid import and assimilate partitioning are regulated by bunch number and leaf area [1], [2] and consequently influence wine yeast metabolism and therefore wine quality. Leaf area and bunch number can be adapted by hedging and bunch thinning respectively. Combining both practices leads to certain leaf area/ fruit mass ratios (LAF-ratio) which have to be optimized according to variety and wine growing region. Belgium is a new wine growing region that needs scientific substantiation on their viticultural methods.

In this exploratory research it is investigated if consumer preference of experimental wines shows trends according to LAF-ratio. Consumer preference of experimental wines is investigated by means of quality assessment by merging ranks of an expert consumer panel. Consumer preference is expressed as an utility value by a rank order logit model. The panel is validated by a Pearson rank correlation coefficient and by presenting wines in duplo [3]. According to eventual trends the LAF-ratio interval can be refined in future research. The influence of LAF adaptation on a novel grape quality parameter, yeast assimilable nitrogen (YAN) content, is also investigated.

Utility values showed decreasing trends according to LAF-ratios with a possible optimum around 10 cm<sup>2</sup>/g (Figure 1). High canopies and low fruit masses under Belgian conditions seem therefore disadvantageous for high consumer preference. YAN contents seemed to inversely follow canopy height. The summer of 2018 was however characterized by high temperatures and very low rainfall which requires multiyear repetition of the experiment.



**Figure 1:** utility values of experimental wines in function of their LAF-ratios

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## Can coagulase-negative staphylococci play a role in the safety of fermented meats? Assessment of antibacterial activity and biogenic amine formation.

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Nitrate/nitrite salts are important preservatives that are responsible for the curing of fermented meats, safeguarding their microbiological safety [1]. However, current trends towards clean-label products demand the need for natural alternatives to displace these additives [2]. Bioprotection via specific microorganisms, being part of the microbial consortium of fermented meats, may offer a possible solution [3]. To that purpose, a screening with 332 staphylococcal strains was performed to explore the antibacterial potential of this microbial group. Strains that showed antibacterial were further assessed against *Clostridium botulinum*, a pathogen that could proliferate in fermented meats free of nitrate/nitrite salts [1]. *Staphylococcus sciuri* IMDO-S72 was the only strain exhibiting promising anticlostridial potential. Besides the prevalence of antibacterial activity, the staphylococcal strains were also screened for their production of biogenic amines. These compounds arise from decarboxylation of amino acids, and can compromise food quality if certain thresholds are exceeded [4]. Using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), the incidence of biogenic amine production appeared to be low and strain-dependent. Tyramine and  $\beta$ -phenylethylamine were the most commonly produced amines, marked by a simultaneous production. In general, concentrations were of no concern for food safety, remaining low even after a prolonged incubation. Being free of biogenic amine production and expressing promising antibacterial activity, *S. sciuri* IMDO-S72 may be of interest to use as a bioprotective starter culture in the production of fermented meats.

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## 2-Aminoimidazoles as a potent inhibitor of brewery biofilms

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Currently, it is thought that 90% of all bacteria live within biofilms, which are surface associated communities of micro-organisms that are embedded in a self produced extracellular matrix (1). Biofilms offer the bacteria many advantages compared to the free living mode of life, such as higher tolerance to desiccation and disinfectants (2). Also bacteria that contaminate food companies and breweries form biofilms, which strongly impedes their eradication. Commonly used cleaning and disinfection protocols are therefore often not able to sufficiently remove the microbes present, indicating that novel anti biofilm strategies are highly needed (3).

2-Aminoimidazoles are a class of anti biofilm molecules under development in our lab, that have been shown to prevent biofilm formation of multiple species (4). The aim of this work was to study the preventive activities of 3 in house synthesized imidazoles against a broad array of 12 natural brewery biofilms and benchmark these activities against a diverse set of 18 inhibitors reported in literature. Subsequently, the top 7 inhibitors, including 2 imidazoles, were selected and retested against 12 brewery biofilm models with defined species compositions. A subset of the 3 best inhibitors, including 1 imidazole, was finally retested in combination with common disinfectants H<sub>2</sub>O<sub>2</sub> or peracetic acid against 6 biofilms: the 3 most and the 3 least tolerant against the inhibitors. Our study supports the potential of 2 aminoimidazole compounds as a preventive anti biofilm strategy for food industry and breweries.

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## Session 1 (ZT1)

# Water & soil science

11h15 -12h15

**Chairman: Jeroen Meersmans – ULiège**

Photocatalytic degradation of thiabendazole by using porous organic polymers (POP) under visible light

Alireza Ranjbari (UGent)

Rhizoremediation of an aged-contaminated soil with two Fabaceae: impact on PAHs bioaccessibility

Marie Davin (ULiège)

Land use effects on the geochemical soil properties and their control on organic carbon in volcanic soils, West Java

Sastrika Anindita (UGent)

Silicon dynamics during 2 million years of soil development in a coastal dune chronosquence under Mediterranean climate

Félix de Tombeur (ULiège)

Flash presentations

# INVESTIGATING STREAM WATER TEMPERATURE IN SOUTHERN BELGIUM: ENVIRONMENTAL DRIVERS AND POTENTIAL IMPACT ON A THERMAL SENSITIVE SPECIES (*SALMO TRUTTA*)

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## ABSTRACT

Stream water temperature (WT) is considered as a key ecological factor. Oxygen solubility, organic matter, decomposition rates,... are influenced by WT. Moreover, WT directly influences aquatic species by interacting with the metabolism, growth and survival of species.

In Wallonia (Southern Belgium, 16,000 km<sup>2</sup>), a water level monitoring network of about 140 stations also measures continuous WT taken at intervals of 10 minutes. Continuous monitoring allows detecting extreme thermal events generally harmful to aquatic organisms and time-limited because of high WT variability.

The objectives of this study were :

- To study WT evolution between 2012 and 2018, which are still underestimated due to the lack of regular and continuous monitoring over long periods;
- To demonstrate the importance of having continuous temperature data for understanding and anticipating thermal damage to the aquatic ecosystem with the example of the brown trout (*Salmo trutta fario* L.);
- To study the influence of land cover, topographical, hydromorphological and seasonal parameters on WT.

The results showed a typical yearly sinusoidal evolution of the WT between 2012 and 2018. However, between years, remarkable thermal differences were observed reflecting the meteorological assessment established for the study period. Moreover, our data allowed us to map areas and target periods when temperatures too hot for brown trout are recorded. In addition to this essential information for the management of aquatic environments, the study of environmental factors has shown that shade has a strong impact on river temperature variability. This result provides an objective basis for conservatory management of riparian forest cover.

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## Humic acid coated P fertilizers to improve phosphorus use efficiency in calcareous soils

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Phosphorus mobility and availability in soil are mainly governed by both soil and fertilizers proprieties including soil P-sorption capacity, soil organic matter content, soil pH and P fertilizer solubility. Although, the application of considerable P fertilizer rates in calcareous soils, containing high levels of calcium carbonate, P availability and uptake are extremely affected, which limit crop production in several cropping systems around the world. One approach to enhance P use efficiency and its agronomic benefits is to use humic substances (HS). In this regard, our study aims to assess the impact of P granular fertilizers coated with HS, on plant growth, mineral nutrition and P use efficiency. Two pot experiments were carried out at the OCP R&D Center using di-ammonium phosphate (DAP) coated with 0.5% of HS (extracted from leonardite) and applied at recommended doses on maize and tomato crops grown under sandy calcareous soil conditions (pH 8.0). As results, coating DAP with HS improved maize and tomato shoot dry weight by 6.2% and 16.8 % respectively, compared to uncoated DAP. Likewise, root dry weight (17% and 30%) and leaf chlorophyll content (+5%) were enhanced by HS coating. In addition, the use of HS improved greatly P uptake by 15% and 13% in both maize and tomato plants, which can have a benefic impact on P use efficiency. Under current calcareous soil conditions, the results showed also that HS improved nitrogen, potassium, iron and zinc plant uptake.

**Key words:** *Humic substances, Phosphorus use efficiency, calcareous soil, maize, tomato*

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## **“Microbial communities are influenced by different soil management practices such as minimum tillage and plowing in historical field trials”**

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Soil is probably one of the most complex environments on the Earth including a huge microbial diversity. Microorganisms are largely involved in agricultural ecosystem functions. These are determined by soil type and properties (pH, C content, texture...) and environmental conditions (temperature, humidity...). The cropping system used over time is also a major factor. Especially, tillage management practices are known to modulate the microbiome which can influence the plant growth conditions <sup>(a)</sup>. In this study, we investigated the soil microorganisms response to plowing and minimum tillage after long-term (15 years) field trials. The factorial experimental design encompasses a mineral fertilization as third treatment (TMS®, a regulator of microbial activities and organic matter decomposition). Soil samples were collected in June under maize crop and for two depths (0-10 and 15-25 cm). The microbiome was profiled using the molecular tools. After total DNA extraction, the molecular microbial biomass (MMB) was determined (µg of DNA/g of soil) and a ratio corresponding to the fungal-to-bacterial abundance was calculated (F/B ratio). The bacterial and fungal diversities were characterized based on the operational taxonomic units after sequencing of the 16S and 18S rRNA gene amplification products, respectively. As assumed, stratification in minimum tillage was observed, with MMB and F/B ratio higher on surface but lower in depth, compared to the plowing practice. On average a higher bacterial diversity (not for fungi) was observed in plowing with slight differences found with depth. Finally, a significant increase in the complexity of interaction networks within the fungal-bacterial community under the amendment was also observed.

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# Microbial communities in a metal- contaminated soil and probable interactions between new isolates

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## Abstract

The present study was focused on investigating a microbial community in a metal-contaminated soil using shotgun metagenomics to highlight the most important metal resistance systems and studying the probable interactions between new isolates in biofilms. The selected soil, located in Aubry (North of France), is contaminated by heavy metal rich dusts (Zn, Cd, Cu, Pb) which deposited in the soil for more than 150 years. A total of 33,808,298 reads of  $252 \pm \text{bp}$  were obtained by the Illumina sequencing technology. After analysis by the MG-RAST platform results indicate that 7 bacterial genera represent 25% of the reads. These genera are *Candidatus Solibacter*, a member of the *Acidobacteria* (9.4% of the reads), followed by *Gemmatimonas* (4.6% of the reads), *Streptomyces* (2.9%), *Chitinophaga* (2.7%), *Nitrospira* (2.0%), *Burkholderia* (1.9%) and *Conexibacter* (1.9%). The most abundant metal resistant system was cation efflux system (67 061 reads). Most of these zinc resistance systems were produced by *Candidatus Solibacter* (12329 reads), *Pseudomonas* (5703 reads) and *Rhodopseudomonas* (5122 reads). Two series of pure bacterial strains were obtained: 10 LMR strains (Low Metal Resistance strains), unable to grow at 1.5 mM Cu; and 6 HMR strains (High Metal Resistance strains) able to grow when Cu concentration higher than 1.5 mM. All the obtained strains were combined (one HMR strain+ one LMR strain), and the biofilms that formed on the walls of the containers were then analyzed using ITS profile, DGGE. One combination of *Cupriavidus*

sp. strain 11 and *Bacillus* sp. strain 27 was able to grow in 1.5 mM Cu. The growth of strain *Bacillus* sp. strain 27 was probably due to the interaction with *Cupriavidus* sp. strain 11 in biofilms.

## Small-scale spatial and temporal dynamics of a reef fish community in the Galapagos

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Identifying the factors that shape fish communities over broad spatial and temporal scales has been a major objective of ecological studies, but small-scale spatial and temporal dynamics and drivers remain largely unknown. In this study, video transects were used to assess how the structure of fish communities at a small spatial and temporal scale are affected by habitat characteristics, water conditions and spatial and temporal dependencies. The physical habitat in combination with spatial auto-correlation, were most important to explain biological differences ( $R^2 = 0.345$ ). Water conditions showed a strong tidal trend, while differences between days were limited. However, different days were more important to explain observed differences in the structure of fish communities ( $R^2 = 0.098$ ) than tidal dynamics and water conditions ( $R^2 = 0.039$ ). These results indicate that at a small scale, fish communities are highly associated to specific physical habitats but rather independent of tidal dynamics and/or water conditions. Being aware of these small-scale variabilities is crucial to successfully identify the drivers that shape fish communities and allows for a more informed sampling design.

## Photocatalytic degradation of thiabendazole by using porous organic polymers (POP) under visible light

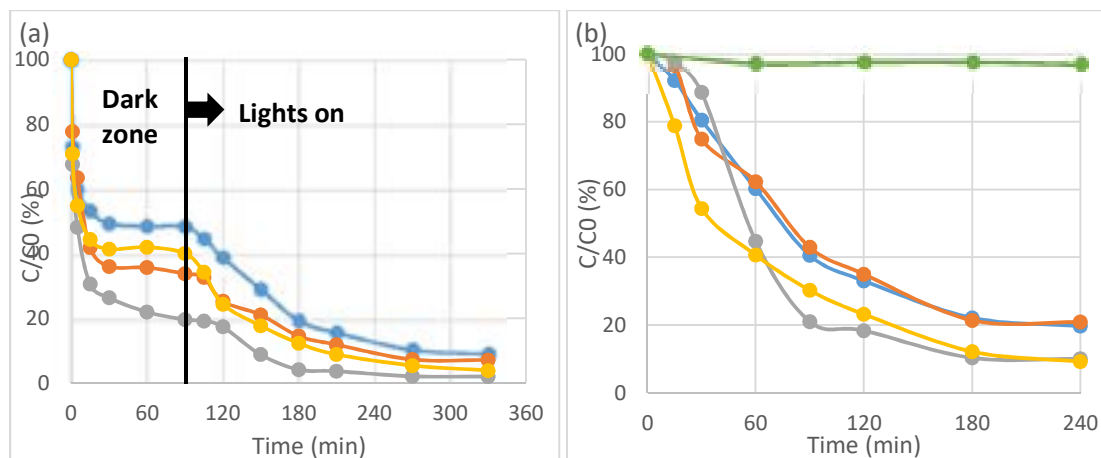
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Thiabendazole has gained a wide application as a fungicide for different plants such as rice, tobacco, sugar cane, tomato, and fruit trees. Also, it has been used for treatment of hepatic diseases in goats and sheep [1]. The residues of this pesticide have been detected in agricultural run-off or food processing industries' wastewater effluent. This can induce harmful effects on the aquatic environment because of its bio-activity and persistence [2].

Semiconductor photocatalysis has been considered as a green and sustainable technology for wastewater treatment. However, application of metal oxides or other metal complexes as photocatalysts is confronted with some limitations such as improper bandgap for visible light excitation necessitating UV-lamps, metal leaching, high price, and chemical instability. Hence, a new class of materials, which are known as porous organic polymers (POPs) are introduced in various fields with applications as adsorbents, storage materials, versatile reaction catalysts, and catalytic solid supports [3]. These novel POPs are tailored  $\pi$ -conjugated organic networks with nanoporous three-dimensional characteristics that have a high surface area and a great chemical and thermal stability. Recently, they have also been developed to resolve the drawbacks reported with common photocatalysts [4].

In this research, the potential of POP-1 [4] for visible light induced photocatalytic degradation of thiabendazole is investigated at different conditions of pH (3, 5, 7, 9), POP and thiabendazole concentrations. Experiments were set-up in two phases: a dark period of 90 min during which adsorption-desorption equilibrium could be established in the water-catalyst system; followed by a 6-hours exposure to visible light (wavelength 400-700 nm) for subsequent thiabendazole photodegradation.



**Fig.1. (a) Relative concentration of thiabendazole versus time in both the dark and illuminated period, (b) Normalized concentration profile of thiabendazole during light-induced degradation, with  $C_0$  the concentration measured after the dark adsorption period. The POP and initial thiabendazole concentration is  $80 \text{ mg.L}^{-1}$  and  $10 \text{ mg.L}^{-1}$  respectively. Light intensity  $122 \text{ W/m}^2$ , temperature  $25^\circ\text{C}$ , reaction volume  $25 \text{ mL}$ , and  $\text{pH} = (\bullet) 3.25, (\circ) 4.8, (\bullet) 6.95, (\bullet) 9, (\bullet) \text{blank}$ .**

Fig. 1(a) shows that neutral pH is the most favorable for both thiabendazole adsorption and photocatalytic degradation. Adsorption results in a removal of 80% after 90 min, which is increased up to 98% removal after an additional period of 240 min photocatalytic degradation under visible light irradiation. Fig.1 (b) shows the normalized photo-degradation profile that compares the reaction progress at each pH regarding the concentration of thiabendazole obtained at the end of 90-min dark adsorption. Despite that thiabendazole is present at the lowest concentration at pH 7 at the start of photo-degradation phase, it still shows the fastest degradation rate at these neutral conditions. In the blank experiment in presence of visible light and without catalyst, it has been seen that there is almost no photolysis occurs at pH 7 after 4 hours of irradiation.

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## DEVELOPMENT AND VALIDATION OF A UHPLC-ESI-MS/MS METHOD FOR DETERMINING ESTROGENIC COMPOUNDS IN SURFACE WATER AT THE ULTRA-TRACE LEVEL

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Natural estrogens (estrone: E1, 17 $\beta$ -estradiol: E2, estriol: E3) and synthetic estrogen (17 $\alpha$ -ethinylestradiol: EE2) are powerful endocrine disruptors harmful to aquatic wildlife and human at extremely low concentrations. The European Commission included these molecules in the first (2015) and second (2018) surface water Watch List issued under the Water Framework Directive regarding emerging aquatic pollutants, proposing maximum detection limits (LOD) of 0.035 ng/L for EE2 and 0.4 ng/L for E1 and E2. Attaining these limits represents a challenge even with the most up-to-date analytical tools, in particular in surface water. A two-step sample preparation, involving a preliminary extraction of a whole water sample on a solid-phase extraction (SPE) disk and further purification on a Florisil SPE cartridge, was optimized. The cleaned-up extract was then quantified by LC-MS/MS following a derivatization step, which greatly enhanced the response in electrospray ionization positive mode (ESI (+)). This derivatization procedure allowed to obtain at least two transitions for each analyte which were proved to be specific and hence selected for quantification and confirmation. The main goal was to maximize the recoveries in order to achieve the very low LODs required by the European Watch List. The method was fully validated in seven surface water. The LODs calculated for all the seven surface water were below the recommended maximum acceptable levels required by the European Commission.

Rhizoremediation of an aged-contaminated soil with two *Fabaceae*: impact on PAHs bioaccessibility.

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<sup>1</sup> These authors contributed equally to the work.

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic compounds that tend to accumulate in the environment. Because of their potential (geno)toxicity and heavy presence in former industrial areas (Keith 2015), PAHs have been the centre of many remediation studies over the past decades.

As an alternative to environmentally aggressive, expensive and often disruptive soil remediation strategies, experiences have been carried on to understand and develop techniques based on bioremediation and phytoremediation, which rely on the use of living microorganisms or plants to remediate pollutions in soil.

Because it is now well-known that the most important limiting factor to PAHs bioremediation is their bioaccessibility (Johnsen et al. 2015), the objective of the study was to investigate the effect of two plants (*Medicago sativa* L. and *Trifolium pratense* L.) on PAHs bioaccessibility in an aged-contaminated soil throughout a long-term rhizoremediation trial.

Therefore aged-contaminated soil samples were cultured with each plant-type, in pots, for 3, 6 and 12 months, and compared to untreated soil. Each modality was repeated five times. Bioaccessible and residual PAHs contents were quantified in soil samples at the end of each culture period using High-Performance Liquid Chromatography with Fluorimetric Detection (HPLC-FLD).

The general rhizoremediation results show that (i) *M. sativa* L. plants developed better than *T. pratense* L. plants on the contaminated soil, (ii) when plants are small or absent, PAHs residual contents globally dissipate faster from the rhizosphere, and bioaccessible contents increase a little faster.

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## Effect of (pyrrolidine-2,2-diyl)bisphosphonic acid on *Raphanus sativus* and *Trifolium repens* : first observations

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Linear biphosphonic acids have already been reported to have herbicidal properties. The purpose of these preliminary tests was to verify the effect on radish and clover plants of the (pyrrolidine-2,2-diyl)bisphosphonic (1) acid, having a nitrogenic cycle.

The first observations upon the use of a solution containing (1) show that watered and sprayed on radish sprouts show a higher stem growth rate but the tested clovers, sprayed on, died within a week.

In conclusion, it seems that spraying the product over the leaves and watering the soil with tap water have more effect, may it be in a good or bad way, than just applying the product in the soil. (1) seems to have a fertilizing effect on *Raphanus sativus* but a herbicidal one on *Trifolium repens*.

## **Soil aggregation stabilizes century old pyrogenic organic carbon in cultivated soils through organo-mineral interactions**

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**Soil aggregation** **Organo-mineral interaction** **Carbon sequestration** **Biochar** **Water & Soil Science** Increasing soil organic carbon contents is amongst our times major challenges for its crucial role in both soil fertility and climate change mitigation. Our study aims to understand the effects of century-old charcoal on the soil aggregation patterns and the stabilization of organic matter. To this end, we focus on century-old pyrogenic organic carbon (PyOC) that was incorporated in soils as residues from pre-industrial charcoal production sites. The accumulation of PyoC in the topsoil of conventionally farmed agricultural field results in a nearby twofold increase in the carbon stock in these relic charcoal hearths as opposed to the nearby uncharred soils. We performed a particle size-density fractionation on soil samples enriched in charcoal and the nearby reference Luvisol. Our objectives were i) to determine the relative distribution of soil fractions, ii) to quantify the carbon content distribution amongst them and iii) to characterize the organic matter continuum using differential scanning calorimetry (DSC). Results showed that macroaggregation is favored at the expense of microaggregation (Char= 25 = 8, Ref= 36 = 8 g 100g<sup>-1</sup>) in charred soils. Moreover, a thermal analysis revealed the presence of PyoC in all soil fractions. When combined with an elemental analysis, an increased H:C ratio of the most physically protected PyOC suggested a functionalization of charred particles thus supporting the assumption that charcoal particles play a role in aggregation. We conclude that century-old Pyoc, beyond its intrinsic recalcitrance, is broken down from coarse to fine carbon pools, and is further stabilized by organo-mineral interactions and aggregation thus contributing to a long-term carbon stabilization in conventional cultivated soils.

## **Silicon dynamics during 2 million years of soil development in a coastal dune chronosequence under Mediterranean climate**

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<sup>4</sup> The University of Western Australia, Australia

Silicon (Si) in plants confers a number of benefits, including resistance to herbivores and water or nutrient stress. However, the dynamics of Si during long-term ecosystem development remain poorly documented, especially the changes in soils in terms of plant availability. We studied a 2-million-year soil chronosequence to examine how long-term changes in soil properties influence soil Si pools and plant-available Si. The chronosequence exhibits extreme mineralogical changes – from carbonate-rich to quartz-rich soils – where a carbonate weathering domain is succeeded by a silicate weathering domain. Plant-available Si concentrations were lowest in young soils (Holocene, <6.5 ka), increased in intermediate soils after the depletion of carbonates and during the formation of clay minerals and Fe oxides (Middle Pleistocene, 120 ka), and finally decreased towards the oldest, quartz-rich soil (Early Pleistocene, 2 Ma). Silicon availability is buffered at low concentrations in the first stages of pedogenesis, likely because of proton consumption by weathering of carbonate minerals and relatively high Si adsorption at alkaline pH. Silicon availability in older soils appears to be controlled by kaolinite dissolution and Si desorption from Fe oxides. The increasing accumulation of biogenic silica following carbonate depletion indicates stronger soil–plant Si cycling as ecosystem development proceeds. A literature analysis confirms the shift in processes controlling Si availability between the carbonate and silicate weathering domain. Long-term pedogenesis imply marked shift in controls over Si availability. This may have important ecological implications in terms of pathogen distribution, regulation of water or nutrient stress, nutrient cycling, and legumes vs sedges/rushes dominance.

Land use effects on the geochemical soil properties and their control on organic carbon in volcanic soils, West Java

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Soils derived from volcanic ash materials are acknowledged to have a higher capability to retain organic carbon (OC). This ability is due to the presence of amorphous materials, such as poorly crystalline minerals and sesquioxides. However, due to their favorable soil properties, volcanic soils in Indonesia are more susceptible to land use change especially from forest to agricultural land. Under agriculture, minerals can weather faster than in natural vegetation and this also impacts soil functioning. We aim to assess the impact of land use on physicochemical and mineralogical soil properties across soils of different age. Current results showed that land use management led to mineral transformation. Pine forest and agricultural land displayed a higher weathering degree than natural forest, as indicated by higher clay content and iron crystallinity index, low weathering index  $(K+Ca)/Ti$ , and the presence of gibbsite. Land use conversion also altered chemical properties such as pH, CEC, basic cations, and the proportion of amorphous materials. Organo-metal and organo-mineral complexes may control OC retention, indicated by ratio  $Al_p$  and  $Al_o$ . Total OC content in surface soil of agricultural land was generally higher than forest, probably due to high organic fertilizer inputs. A significant proportion of carbon is stored in the sand fraction in agricultural land at young and intermediate soil aged, while it is more readily found in silt and clay at old soil age.

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## **Is least limiting water range a useful indicator of the impact of tillage management on maize yield?**

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<sup>2</sup> Chinese Academy of Agricultural Sciences, China

Tillage management is a key factor driving changes in soil physical properties (SPP) and crop yield around the world. However, there is a lack of knowledge about the relationships between SPP and crop yield. The dynamic of SPP during the growth period is also seldom taken into account to understand suitable soil physical environment for crop growth. Moreover, the crop growth process cannot be explained by an individual SPP substantially. The least limiting water range (LLWR), which integrates soil penetration resistance, air porosity, and soil water potential, may provide a better understanding of soil-crop relationship, especially in regions with limited precipitation. Our objective was to explain how dynamic SPP affected grain yield during the growth period. A long-term field experiment was established in 2003, with continuous spring maize, on sandy loam soil. Seasonal changes of SPP (i.e. bulk density, penetration resistance, porosity, mean weight diameter, LLWR, and plant available water) were determined under reduced tillage with residue incorporated (RT-RI), conventional tillage with residue removal (CT), and no-tillage with residue mulch (NT-RM). The results showed that these SPP were affected by both tillage management and growth stage. Bulk density, porosity, S index, and mean weight diameter were not effective indicators to explain the changes of grain yield under the three tillage managements. The range of LLWR was narrower than plant available water (PAW) during the growth period and more sensitive to assess soil water availability under RT-RI, CT, and NT-RM. NT-RM significantly increased the lower limit of LLWR, which made it more difficult for root water uptake. Hence, RT-RI presented higher corn yield compared to NT-RM, even if the water content remained lower. Redundancy analysis further indicated that maize yield was mainly driven by the lower limit of LLWR and penetration resistance. Overall, LLWR was an aggregative indicator including not only soil penetration resistance but also air porosity and soil water potential, which can better explain the change of grain yield under the long-term tillage management in semi-arid region.

## Increase of microorganisms in macroaggregates promotes carbon turnover after straw application

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Change in organic substrate after the application of straw, directly induce variations in the metabolism of soil microbial communities and the carbon fixation in soil. Aggregates are basic soil units with heterogeneity inside, and the microbes distribution and activity within or between the aggregates are different, undergoing metabolism activities simultaneously. Generally, the carbon sequestration process is completed by the combined effect of different aggregates and different microbial communities, and the contribution of segregated aggregate groups including the internal microorganisms to the carbon sequestration process cannot be observed. Understanding the mechanism of the effect of microorganisms on carbon inside different aggregates will be of great help in exploring soil carbon fixation. In order to achieve this goal, a combination of long-term test and short-term incubation were used in this study. The former was used to confirm the effect of aggregates on carbon fixation after straw application. The latter is to observe the effect of independent incubation of aggregates on carbon fixation. The direct determinant role of microbial activity on carbon fixation was determined through sterilization and non-sterilization during the cultivation. The <sup>13</sup>C-labeled straw was used to distinguish the new carbon fixation and the decomposition from old carbon by the microorganisms. At the same time, the variations on the microorganisms community during process was observed to determine the impact of microorganisms on carbon fixation in different aggregates. The results showed that the trend on carbon fixation by the aggregates in the incubation was consistent with the results of the long-term test, indicating that the microorganisms in the aggregates had a major role in carbon fixation. It was also found that macroaggregates have larger space on carbon- fixation than microaggregates, and that fungal, negative bacterial and actinomycetes play major role in macroaggregate, and the microenvironment tends to be bacterial. The difference was that although a small amount of new carbon could be observed in microaggregates, the total carbon did not change significantly, indicating that the carbon in microaggregates was in a dynamic change, in which negative bacteria and actinomycetes play a leading role.

## Session 2 (ES)

# Sustainable crop production & protection

14h00 -15h00

**Chairman: Xavier Draye – UCLouvain**

Assessing Grassland Use Intensity using Sentinel-1 Data for Biodiversity Monitoring  
Mathilde De Vroey (UCLouvain)

High-throughput testing for NUE-related traits: microphenotyping of root development and bioassay of root metabolic activity of *Arabidopsis thaliana* plantlets  
Laurence Galhaut (ULiège)

Collaborative design of a sustainability diagnosis based on simple in-field measurements for Walloon field crop systems  
Lola Leveau (UCLouvain)

Biological activities of some Plant-Associated *Bacillus* sp. and *Paenibacillus* sp. upon growth in root exudates of Maize (*Zea mays*) and Tomato (*Lycopersicon esculentum*) varieties cultivated in Democratic Republic of Congo (DRC)  
Virginie Korangi (ULiège)

Flash presentations

## Factors driving cereal response to fertilizer microdosing in sub-saharan Africa: A meta-analysis

Yacouba Ouedraogo<sup>1,2</sup>, Jean-Baptiste Sibiri Taonda<sup>2</sup>, Idriss Sermé<sup>2</sup>, Bernard Tychon<sup>3</sup>,  
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Fertilizer microdosing (FM) is being promoted in sub-Saharan Africa (SSA) to boost crop productivity on smallholder farms. This technique consists in applying small amounts of mineral fertilizer (2 to 6 g) in the planting holes at planting or next to the seedlings after emergence. However, yield response variability is a barrier to adoption. We conducted a meta-analysis to analyze the variability in cereal crop yield response to FM and to determine the main factors associated with this variability. Thirty publications pertaining to millet (*Pennisetum glaucum* (L.) R. Br.), sorghum (*Sorghum bicolor* L. Moench) or maize (*Zea mays* L.) were assessed. Factors analyzed were crop type, rainfall, soil texture, type and rate of fertilizer, and complementary practices. On average, FM improved millet, sorghum and maize crop yields by 68%. Yield response tended to increase with increasing rainfall. The largest yield gains were observed in medium-textured soils (81%) as compared to light (61%) and heavy-textured soils (30%). The combined application of N and P performed better than either element alone. Crop response tended to increase with increasing rates of N irrespective of soil type. In the case of P, this was true only on light textured-soils. On medium-textured soils, the response appeared independent of the rate of P. There was a synergetic effect of water conservation measures on the performance of FM, while combining FM with organic amendments decreased its performance. Results highlighted major trends in cereal crop response to FM that could be used to prioritize target areas. However, these may require additional, site-specific field experiments, especially for factors for which little data is currently available.

## **Study of the evolution of microorganisms communities in an aquaponic system over the course of a full lettuce growth cycle**

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Aquaponics is a soilless crop technique which enables the production of fresh fish and vegetable while saving water and mineral fertilisers. The functioning of this innovative technology is based on a fragile equilibrium between 3 groups of living organisms i.e. fish, plants and microorganisms. Little is currently known about microorganisms in aquaponic systems and even less when it comes to their evolution over the course of several weeks. The aim of this work is thus to thoroughly study the microorganisms communities living in an aquaponic system of Gembloux Agro-Bio Tech throughout a full lettuce cycle, in real conditions. To this end, samples were collected from four different compartments of the aquaponic system (sump, biofilter, roots' rhizoplane and roots' endosphere). High throughput sequencing was then used to sequence microorganisms' DNA and bioinformatics tools are then used to assign taxonomic identification. The main results are that in each compartment, the microorganism community seems resistant to water parameters changes occurring over the course of a full lettuce cycle. However, a drastic modification has been observed after a few weeks in the biofilter and root communities. Indeed, these communities were at first dominated by the *Lactobacillus* and *Streptococcus* genera, already present on the roots and in the biofilter before the seedlings transplantation. Further tests are currently ongoing to identify the cause of this transition.

## Linolenic fatty acid act as potential biocontrol agents and elicitors

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Nowadays, biopesticides have emerged as a main alternative to conventional agriculture. In this context, plant oxylipins, a vast and diverse family of secondary metabolites originated from polyunsaturated fatty acids (PUFAs), appear to be crucial agents in plant defence mechanisms. Actually, it is highly known that plant oxylipins are produced under a wide range of stress conditions. While those molecules are well known to activate several signalling pathways and to induce adaptations in plant exposed to (a)biotic stresses, non-signalling roles of phyto-oxylipins are poorly understood. Among plant oxylipins, the 13-hydroperoxy oxylipins (13-HPO) constitute key intermediate oxylipins (KIOs) as they can be converted into jasmonic acid, OPDA, dn-OPDA or traumatic acid, well-characterized

components involved in plant resistance mechanisms [1][2]. Their presumed functions include direct antimicrobial effect, stimulation of plant defence gene expression, and/or regulation of plant cell death [3]. However, the precise contribution of each of those molecules in plant defence remains unknown.

In this study, 13-HPO properties as direct biocidal agents are investigated. In vitro assays have showed that KIOs can hinder growth of some plant microbial pathogens, with differences between strains and KIOs forms. Further investigation are needed to know if they maintain this power while being exogenously applied on plants, before or after infection.

Afterwards, this study aims to understand the oxylipins action mechanisms and especially their membrane activities. As KIOs are found to be potential biocontrol agents and also to interact with plant plasma membranes [4], their interactions with plants and pathogens plasma membranes were studied using biomimetic membranes via a complementary in silico informatics and in vitro biophysical approaches.

Finally, in analogy with other amphiphilic molecules (e.g. surfactins), KIOs may act as elicitors. This hypothesis is reinforced by results showing the production of reactive oxygen species (priming agents of eliciting reaction) when tobacco roots and leaves were in presence of KIOs. Further investigation are needed to confirm this property and to determine the mechanism of action behind.

## **Production and optimizing a Slow Release Fertilizer based on cotton straw biochar and NPK**

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Most of soils in Burkina Faso are deficient in nutrients due to inherent proprieties and inappropriate agriculture practices. Mineral fertilizer is the maint practices to improve sustainable yields. However, their high costs combined with inefficient application techniques reduce their effectiveness and limit their use by small farmers. The current challenge is to propose sustainable alternatives to optimize mineral fertilization in order to improve agricultural production and the resilience of small farmers. This study aims to develop and evaluate the performance of a slow release fertilizer based on biochar and NPK.

We assessed nutrient adsorption capacity on biochar activated with three levels of NPK; T1=100 kg/ha, T2=125 kg/ha and T3=150 kg/ha. The total N, P, K Ca and Mg adsorbed by biochar were evaluated through chemical analyses. P, K, Mg and Ca release dynamics were monitored by a sequential leaching test in distilled water and Calcium Chlorure (0.001M) at 2, 4, 7 and 14 days.

The results showed that nutrient retention capacity of biochar varied proportionally with NPK doses used to boost it. Biochar adsorbed nearly 992% of the nitrogen supplied by the NPK. More than 50% adsorbed nutrients are released in the first week of the experiment. This study showed that biochar could be used as a nutrient carrier to create Slow Release Fertilizer (SRF) and improve mineral fertilizers efficiencies in tropical wheathering soil.

## Networks of Interactions in Intercropping

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### Abstract

Intercropping (IC) consists in growing various crops on one single plot at the same time. Considering the wide range of advantages (i.e. higher yield, reduced needs of pesticides and weeds management (Mommer et al. 2010; Lin 2011)), IC is now seen as one possible way to improve farming sustainability. However, adoption of IC in Belgium remains low. In practice, IC is more difficult to manage than pure crops and the degree of performance usually displays a large variability (Malézieux et al. 2009; Lemken et al. 2017). It is known that many interactions can occur between associated crops, which can lead to either facilitation or competition. However, the exact relationships leading to facilitation or competition and the conditions in which they occur are still unclear. Consequently, it is hard to predict the outcome of a given IC design in a given environment and to advice farmers. In order to gain understanding about how IC works and to highlight gaps in current knowledge, we made an extensive literature review whose aim was to list all the interactions, - more than a thousand so far -, that are claimed to take place between two associated crops and their environment. We then used the results of this review to create networks of interactions in IC based on a new ontology.

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## DYNAMICS OF ASCORBIC ACID CONTENT IN APPLE (*MALUS X DOMESTICA*) DURING FRUIT DEVELOPMENT AND STORAGE

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Vitamin C is a crucial antioxidant and cofactor for both humans and plants. Unfortunately, apple fruits that are currently available for consumption contain low amounts of vitamin C, making vitamin C an interesting trait for apple crop improvement. With the aim of breeding high vitamin C apple cultivars it is important to get insights into the natural biodiversity of the vitamin C content in apple fruits.

In this study, we have monitored the vitamin C content in the pulp of 79 *Malus x domestica* accessions at harvest. Overall, there was a strong variation in ascorbic acid (AsA), dehydroascorbic acid (DHA) and total vitamin C (AsA + DHA) content between cultivars, indicating a significant genetic variation. A population structure analysis, using an 8K SNP chip, revealed that local/non-commercial accessions display a bigger variation in total vitamin C content in the pulp compared to elite accessions. Local cultivars also contained higher amounts of DHA than elite cultivars, although no significant differences in total vitamin C content were observed.

Out of the 79 screened apple cultivars, the ten genotypes with the highest and lowest concentration of vitamin C were selected for a thorough analysis of the vitamin C dynamics during fruit development and storage. For all ten cultivars and at any point during development and storage the apple peel contained a higher level of vitamin C than the pulp, most likely because the peel is directly exposed to (a)biotic stresses. Interestingly, during fruit development, the AsA/DHA ratio increased in both apple pulp and peel, while throughout storage the AsA/DHA balance shifted to the oxidized form, putatively reflecting a reaction to abiotic stress.

Overall, our study provides a fundamental platform for breeding high vitamin C apple cultivars and for developing novel strategies to maintain vitamin C levels during storage.

## Enrichment in C3 of *Hermetia illucens* prepupae of from oilseed co-products

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Belgian rainbow trout farmers from the Wallonia are currently looking for alternative feed sources. Insect mass production could be an interesting source of alternative protein and fat for fish production. Among the available species panel, *Hermetia illucens* (L. 1758) is a promising species with a polyphagous diet, a fast larvae growth rate and a short life cycle. The current composition of *H. illucens* larvae is characterized by a high-saturated fatty acids level with mainly lauric acid. The objective of this study was to adjust the profile in polyunsaturated fatty acids of the larvae by manipulating their diets. Considering the seasonality and low-fat content of local plant co-products, the diet formulations were supplemented with oilseed meal cakes. After selecting populations (100 individuals/population) of *H. illucens* larvae ( $\pm 0.01$  g), diets enriched with flax and 2 rapeseed meal cake (chemical vs mechanical extraction) were tested in a controlled environment (60% relative humidity - 27 °C). 6 incorporation rates per meal cake (10 - 20 - 40 - 60 - 80 - 100%) were tested in triplicate (n = 3). The larvae were collected at prepupal stage, lyophilized and ground. AGs of prepupae flours were extracted by Folch's method and chromatographed. Protein and ash levels were also analyzed. The proportions between saturated fatty acids (SFA) and unsaturated fatty acids (UFA) were evaluated as well as the percentage of  $\alpha$ -linolenic acid (ALA - C18: 3C3). The incorporation of meal cake has reduced the ratio SFA/UFA in proportion of 4,11 to 1,44 and increased ALA percentage of 0,72 to 15,83. The increase in the proportion of unsaturated fatty acids in *H. illucens* prepupae flour should allow an increase in the incorporation rate of artificial diets formulated for rainbow trout.

## AQUAPONIC WATER, A SUPPRESSIVE SOURCE OF BIOCONTROL AGENTS AGAINST *PYTHIUM APHANIDERMATUM* ROOT ROT IN LETTUCE

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### Abstract

In aquaponics, phytosanitary treatments for plant diseases management are a delicate matter because of the presence of fish. However, it appears that aquaponic systems could be naturally armed against plant pathogens. This natural protective action is called suppressiveness. Aquaponic water has shown a direct inhibitory effect on *in vitro* *Pythium* spp. growth (Gravel et al. 2015; Sirakov et al. 2016; Stouvenakers et al. 2018). To confirm this discovery, *in vivo* experiments using *Pythium aphanidermatum* have been carried out on lettuces growing in hydroponic (HP) water, aquaponic (AP) water or aquaponic water complemented (COMP) with mineral nutrients to reach HP levels. Suppressiveness property of AP, COMP and HP waters was evaluated by comparing root symptoms and lettuces yields. For each treatments, lettuces root microbiota were analysed after Illumina high throughput sequencing of ITS and 16S rDNA genes. Results showed that yields and roots health of AP lettuces were significantly better compared to COMP and HP lettuces. The bioinformatics analysis of sequenced microbiota showed differences in terms of microbiota diversity and features composition when comparing AP with COMP and HP water. These results highlight that the physicochemical modifications of the AP water to make the COMP water disturb the AP microbiota and lead to the loss of its suppressive capacity. Moreover, AP could be an interesting source of novel biocontrol agents for plant pathogens control in aquaponics.

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## Changes of feeding behavior and salivary proteome of Brown Marmorated Stink Bug when exposed to insect-induced plant defenses

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The invasive Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* Stål, has dispersed widely throughout North America and Europe, negatively impacting agro-ecosystems and urban areas. This species is phytophagous and highly gregarious at all developmental stages. Therefore, it is important to determine how the congeners react to plant defenses induced by first infestation. Lipxygenase activity was found to be enhanced in faba bean (*Vicia faba* L.) leaves by BMSB feeding or its salivary compounds. We analyzed BMSB feeding behavior by comparison with our previously published EPG waveform library for that pest, and identified some EPG variables associated with test probes, stylets pathway, and sustained ingestion. We demonstrated that, on elicited plants, BMSB probes were delayed, with sustained ingestion events being shorter. Moreover, significant changes in salivary gland proteins involved in plant allelochemical detoxification were detected when BMSB was exposed to plant defenses. Our results confirmed that this polyphagous invasive Heteroptera has the ability to detect plant defenses and to adapt its feeding strategies in consequence.

## **Exploring the virus richness and diversity in contrasted plant communities of Poaceae in Belgium**

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Prior to plant domestication, it is hypothesized that plant viruses were co-evolving with uncultivated plants growing in mixed species communities, thereby resulting in complex interactions (antagonism, commensalism, mutualism). Development of agriculture further deeply modified natural ecosystems, land use and dynamics of virus-plant interactions, which has fostered occurrence of virus disease emergence events. In this context, we are conducting a study in the Natural Park “Burdinale-Mehaigne” (Belgium) using high throughput sequencing technologies in order to examine the impact of plant species diversity on the virome (e.g. the genomes of viral community) of Poaceae in contrasted plant communities (cereal monocultures, grazed pastures and natural grasslands).

We adapted a virion-associated nucleic acids (VANA) metagenomics protocol to sequence at high throughput pools of 50 plant samples per plant community (50 samples reflecting plant species composition) and per plant species. Over two years, about 4,300 Poaceae plants (corresponding to 24 species) were sampled and bioinformatic analyses revealed presence of diverse viral communities in wild and cultivated Poaceae, even though they did not present any symptoms. These viruses belong to diverse families (e.g. Alphaflexiviridae, Luteoviridae, Partitiviridae, Totiviridae), infecting a large range of hosts within Poaceae and transmitted by different vectors (insects, mites, nematods) or seed-borne. More than 30 virus species were detected in the different plots and at least half of them are candidates for new virus species, revealing that virome in Poaceae-based agroecosystems and its impact on the dynamic of plant communities remain largely unexplored.

## Root fungal community structure of *Alkanna tinctoria* differs with plant developmental stage

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### Abstract

*Alkanna tinctoria* (Boraginaceae) produces alkannin/shikonin (pharmaceutical substances with a wide spectrum of biological properties) and growing evidence suggest to date that endophytes (i.e. bacteria and fungi) are beneficial to plant growth and secondary metabolites (SM) production. Since almost nothing is known about the root fungal community structure of this plant, there is a need for a thorough analysis of its fungal community structure per developmental stage, allowing identification and isolation of promising microorganisms for future applications in SM production systems. We characterized the fungal community structure of *A. tinctoria* with Amplicon Sequence Variant (ASV) and diversity (Simpson) index by Illumina MiSeq sequencing based on the ribosomal ITS region. The plants were grown under controlled greenhouse conditions, in a mixture of sterilized substrate (peat moss and perlite) and natural (non-sterilized) soil from two locations in Greece (soil A and B). A control that only comprised the sterile substrate was included. The plants were harvested at four developmental stages (I, II, III and IV), corresponding to peak of growth, flowering, fruiting and dormancy, respectively. Based on ASV data, the fungal community diversity of the control plants was significantly lower and different from the those of plants

grown in the two Greek soils, whatever the developmental stage. Similarly, the total fungal diversity in soil B was significantly higher than in soil A, regardless of developmental stages. Finally, differences were noticed between developmental stages and soils. The fungal communities of plants grown in soil A and B were similar at the stage I and IV, while different at stage II and III. In each stage, more than 30% of the fungi were shared between plants grown in soil A and B. A stable core microbiome (i.e. present at all developmental stages) was identified. In soil A, a total of 45 ASVs (16%) were present at the four stages examined and in soil B, 51 (18%). By merging these two results, 31 ASVs were always occurring in the roots of *A. tinctoria*, regardless the soil and developmental stage. This study reports for the first time the root fungal community of *A. tinctoria*. A wide diversity of fungi was detected in the root system along the plant developmental stages with a stable core microbiome identified throughout the stages. These results open the door to the isolation and testing of promising fungal endophytes to be applied in SM production systems aiming at high yields.

## Key words

*Alkanna tinctoria*; Fungi community; ASV; stable core microbiome; secondary metabolites

## **Assessing Grassland Use Intensity using Sentinel-1 Data for Biodiversity Monitoring**

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Over the last decade, many efforts have been focusing on providing land cover maps at various scales and with different levels of precision. In some cases, it is however more relevant to identify “land use” rather than “land cover” classes. Grasslands represent a vast and diverse land cover class. In the context of biodiversity assessment, all grasslands cannot be given the same biological value. A distinction should be made between natural grasslands and agricultural grasslands with different levels of intensity. With the increasing availability of satellite imagery with high spatial and temporal resolution, it should be possible to differentiate grasslands in terms of land use intensity and thereby provide valuable information for habitat monitoring and preservation.

This study focuses on Belgium, in the frame of LifeWatch FWB which provides land cover data and numerous other variables for biodiversity research, through the ecotopes dataset. The aim of this study is to discriminate different grassland use intensities based on mowing detections using Sentinel-1 SAR time series. As the RADAR signal is independent of sun light and clouds, Sentinel-1 provides a continuous global coverage with a 6 days frequency. The impact of speckle is reduced by using a parcel-based spatial smoothing. Different mowing detection methods are developed calibrated and compared using field data available over a large number of grasslands for 3 years (2017-2018-2019). Mowing events are detected very accurately in some grassland parcels. In other cases however, detection is difficult due to the parcels’ small size, their slope and orientation or the presence of trees and shrubs. Overall, results are promising and suggest it should be possible to use satellite-based mowing detections to contribute to grassland use intensity assessment.

## Factors shaping the phyllosphere bacterial communities of greenhouse crops

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The phyllosphere is the above-ground surface of plants. Beneficial bacteria on the phyllosphere can inhibit pathogens directly, through microbe-microbe interactions or indirectly, by triggering the plants defense system. Phyllosphere bacteria can also enhance plant growth through the production of plant hormones or by increasing the availability of nutrients. This research aims to gain insights into the taxonomic composition of a healthy phyllosphere microbiome and what factors shape these communities. In this study, the phyllosphere communities from seven tomato and three strawberry cultivars were sampled during eight weeks. 16s rRNA gene amplicon sequencing was used to determine the bacterial community structure of the phyllosphere and its dynamics over time. The bacterial communities were low in diversity and highly variable over time and in space. This is in contrast with the stable greenhouse environment that is highly controlled to minimize biotic and abiotic stresses. These results suggest that the bacteria on the phyllosphere did not reach a stable community. Interestingly, a high amount of bacteria on the leaves originated from bumblebees, used in the greenhouse. Indicating that insects play an important role in shaping phyllosphere communities. These insights in the composition and dynamics of bacterial communities are a crucial step towards understanding and modulating phyllosphere communities to improve crop production and crop protection.

## ABSTRACT

### **Cassava brown streak disease and cassava mosaic disease epidemiologies in farmer's seed systems of the eastern D.R. Congo: trends and determinants**

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Vegetatively propagated crops are particularly sensitive to disease dissemination through the seed system. Strict phytosanitary measures can be instrumental to limit the impact of diseases as illustrated by the potato seed system in Europe.

Our research aims at studying the cassava seed system, a vegetatively propagated staple crop in the tropics, and develop mitigation measures to reduce the impact of viral diseases in cassava-producing regions.

In total, 250 cassava farmers were surveyed and 246 cassava fields sampled in the Uvira territory/eastern D.R. Congo from April to October 2019. Specific social, economic and ecological parameters were used to categorize the study area into sites.

Our analysis indicates that several characteristics of the cassava seed system could contribute to determining the occurrence of CBSV and CMD symptoms in farmers' fields, including the level of education of farmers, the practice of livestock farming activity and their accessibility to information from supporting agents ( $P\text{-Chi}^2 > 0.001$ ). Results further revealed that CBSV incidence in farmer fields was influenced by the characteristics of sites considered ( $P\text{-F} > 0.001$ ), while CMD incidence was depending on the origins of cuttings used for plantation and the severity of CBSD symptoms ( $P\text{-F} > 0.001$ ).

Cross-sectional understanding of local parameters governing the epidemiology of CBSD and CMD are of key importance for developing tools and strategies to implement a robust and sustainable cassava seed system. Linking these findings to the molecular analyses of the cassava virome will be carried out to better understand the epidemiology of these viruses and their association with symptoms.

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**FEVERPRO Project: Development of biological control methods in alternative to pesticides to manage *Bruchus rufimanus* Boheman 1833 (Coleoptera : Chrysomelidae) in field bean crops (*Vicia faba* L. Fabaceae)**

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*Bruchus rufimanus* Boheman 1833 (Coleoptera : Chrysomelidae) is a serious pest of *Vicia faba* L. (Fabaceae) preventing the valuation of crop products due to its post-embryonic development taking place inside forming seeds. However, the European Union, concerned about the sustainable development of its agriculture, promotes *V. faba* crops for its multiple services provided to cropping systems, and restricts at the same time the use of effective pesticides against *B. rufimanus*. This suggests the finding of alternative control methods, such as the use of semiochemicals. To date, some volatile organic compounds (VOCs) involved in the field colonization, feeding, mating and oviposition of *B. rufimanus*, taking place on different parts of *V. faba* and following its phenological stage, have already been identified. Further studies would, however, provide insight into other pheromonal means of communication that could be implemented in the semiochemical-based management of this pest, such as the assessment of oviposition or aggregation pheromones. Concerning the semiochemical-based control methods, only one study has been trying to implement a mass-trapping method; and no studies investigated other methods, such as the push-pull or attract-and-kill methods, in IPM strategy including new insight into pheromone identification, entomopathogenic agents or specific parasitoids. In this context, preliminary experimentations of entomopathogenic tests and odor samplings for the identification of new pheromones were carried out within the “FEVERPRO project”, and could suggest the implementation of innovative control methods of this specific pest in alternative to the use of pesticides.

## Biochar and cocompost: an option for sustainable fertility management of leached tropical ferruginous soils

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The current challenge for agriculture in Burkina Faso is to find innovative options for sustainable fertility management that use accessible technologies to increase agricultural production while preserving the soil. An experiment to recycle nutrients through the production of biochar from cotton stalks was carried out in the village of Massala in western Burkina Faso. The association of biochar with the composting process called co-composting was tested on 03 soil types. The experimental design used is a BCR with 03 treatments and 04 replicates to determine the effects of the different treatments on cotton yield for different soil types. The area of each the elementary parcel was 120m<sup>2</sup>. The 03 soil types are Chromic lixisol, endoplinthic lixisol and epipetric Plinthosol.

The analytical results show that biochar gives the best yield on epipetric plinthosol (932.25 kg.ha<sup>-1</sup>) and endoplinthic lixisol (1081,37 kg.ha<sup>-1</sup>). On the other hand, on chromic Lixisol, with a significant difference ( $p < 0.0001$ ), cocompost gives the best yield (1876,75kg.ha<sup>-1</sup>). The best yield per treatment\*soil type was obtained with cocompost\*chromic lixisol (1876,75 kg.ha<sup>-1</sup>) while compost\*epipetric plinthosol with 769.64 kg.ha<sup>-1</sup> gave the lowest yield.

Furthermore, our results showed that biochar and cocompost improve seed-cotton yield on soils with a low fertility level of 12,5% and 16;61% compared to soils with a medium fertility level.

The transformation of cotton stems into biochar for co-composting improves agricultural production, reduces greenhouse gas emissions and is an option for sustainable soil fertility management.

## **High-throughput testing for NUE-related traits: microphenotyping of root development and bioassay of root metabolic activity of *Arabidopsis thaliana* plantlets**

Laurence Galhaut, Davide Savy, Pierre Delaplace, Patrick du Jardin

Plant Sciences, Terra Teaching and Research Center, Gembloux Agro-Bio Tech, University of Liège, Belgium

Plant biostimulants are a new class of fertilising products, defined as any substance or microorganism applied with the aim to enhance plant's nutrient use efficiency, abiotic stress tolerance, crop quality traits or the availability of confined nutrients in the soil or rhizosphere, regardless of its nutrients content [1]. As the definition of a biostimulant product refers to its action rather than its composition, there is a need to develop tools to evaluate their bioactivity, for screening purposes, for dissecting the mechanisms of action and for validating the effects claimed by the product labels. In this work, we set up laboratory-scale biological tests related to nutrient use efficiency and more precisely to nutrient acquisition, by assessing root architecture and rhizosphere acidification. The first test allows the high-throughput microphenotyping of *Arabidopsis* roots and shoots, in normal or abiotic/nutrient stress condition, using seedlings growing in microplates. Multiple developmental variables are analysed (i.e. root elongation, root branching, mass distribution vertically and horizontally), allowing to discriminate between the effects of even closely related biostimulants. The second test is based on the capacity of roots to acidify the rhizosphere, which is related to respiration intensity, proton extrusion, exudation of organic acids and nutrients uptake. This test uses a pH indicator and colorimetry to monitor the acidification of the growing media of treated *Arabidopsis* plantlets. Changes in the acidification activity, as compared with roots of control plantlets, are regarded as first indication that the substance modulates some root metabolic activity involved in nutrient uptake.

### **Reference:**

1. Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 (Text with EEA relevance)

## Acute toxicity of fungicide-insecticide mixtures on honeybee

Louis Hautier, Justine Gruntowy, Jessica Denayer, Gilles San Martin

CRA-W, Belgium

Bees contribute to crop pollination and must be protected to preserve this important ecosystem service. In the field, bees can directly exposed during the pesticide spraying or by drift on the field edge but also by residues in nectar and pollen in nectar and pollen leading to exposure to a cocktail of residues (Simon-Delso et al., 2014). In addition, tank mixtures are a common agricultural practice to reduce the number of spraying. To date, the risk of pesticide mixture is not considered for bees in Belgium. To fill the gap, we studied the acute toxicity of some potential mixtures of triazole fungicides and pyrethroid insecticides. Oral exposition was made with a contaminated syrup for each product separately and in combination according to the guideline OECD213. After 48h exposition, we observed with the mixture of the insecticide Mavrik (tau-fluvalinate) and the fungicide Prosaro (prothioconazole and tebuconazole) an increase of the toxicity up to 7 times in comparison to alone exposition of each product at the same dose. In reverse, the mixture Mavrik and Citadelle (chlorothalonil and cyproconazole) didn't lead to a synergistic effect. By including the toxicity of product mixtures in the risk assessment, the side effects from these mixtures could be easily reduced for bees.

Simon-Delso, N., San Martin, G., Bruneau, E., Minsart, L.-A., Mouret, C., & Hautier, L. (2014). Honeybee Colony Disorder in Crop Areas: The Role of Pesticides and Viruses. PLoS ONE, 9(7), e103073. <https://doi.org/10.1371/journal.pone.0103073>

## **Stress-related accumulation of arabidopsides: impact on plant chloroplasts**

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Oxylipins are crucial agents in plant defense mechanisms. While free oxylipins are well studied, roles of esterified oxylipins remain unclear. Esterified oxylipins are structurally diverse metabolites that were found in diverse plant species, suggesting that those may be more ubiquitous than currently thought. Among those, galactolipids containing (dn)OPDA were discovered, firstly in *A. thaliana*, but also in other plants. Those molecules, named arabidopsides, are highly induced under stress conditions, as they accumulate up to 8 percent of plant lipids, but their precise contributions in plant defense mechanisms are still unknown. Arabidopsides are directly formed in plant chloroplast membranes from galactolipids. Accumulation of arabidopsides in such high quantity in chloroplast membranes may modify their properties (e.g. photosynthetic activity).

This study aims to understand the impact of arabidopside presence in chloroplast membranes on their properties using biomimetic plant membranes via complementary *in silico* and *in vitro* approaches. Interfacial properties of arabidopsides and non-oxidized galactolipids were studied using Langmuir film balance. Results showed that arabidopsides possess different interfacial properties compared to non-oxidized chloroplast lipids. Arabidopsides' ability to permeabilize chloroplast membranes was also studied *in vitro*. Arabidopsides A and B are able to permeabilize chloroplast membranes while arabidopside D is not.

In conclusion, arabidopside production by plants under stress conditions may modify chloroplast membrane properties such as its permeability. As chloroplast membrane lipid composition is essential to its photosynthetic ability, such changes may also affect its function.

**Effects of light and dissolved oxygen on the growth of three microgreens: *Ocimum basilicum citriodorum*, *Raphanus sativus longipinnatus* and *Brassica rapa cymosa* grown in hydroponic systems.**

Wael Ben Hmouda<sup>1</sup>, Françoise Bafort<sup>1</sup>, Haïssam M.Jijakli<sup>1</sup>

<sup>1</sup>Integrated and urban Plant Pathology Laboratory, Gembloux Agro-Bio Tech, Liège University

The microgreens culture has become a new gastronomic trend in recent years thanks to its colors, textures and flavor that can be served as a garnish or as a new salad ingredient. They are usually harvested when the first pair of true leaves appears or when the cotyledons are fully developed. To produce these microgreens indoors through hydroponics, we are increasingly using artificial lighting and oxygen dissolved in order to improve crop growth and quality. Three plant species: Radish Rape (*Raphanus sativus longipinnatus*) Broccoli (*Brassica rapa cymosa*) 'Barassicaceae family' and lemon basil (*Ocimum basilicum citriodorum*) 'Lamiaceae family' were cultivated in controlled environment chambers using the DWC Hydroponic System (Deep Water Culture).

This study examines the effect of different levels of illumination and spectrum produced by LEDs, and the effect of different concentrations of dissolved oxygen produced by air pumps on the growth of microgreens grown in hydroponics. The light intensity test was performed using four photosynthetic photon flux density (PPFD) of P: 300,200, 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and PS:100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  emitting by another type of LED.

The results showed that 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  treatment gave the best results for the 3 species. The dissolved oxygen test showed that plant growth improves with increasing oxygen concentration in the nutrient solution.

## Conservation Agriculture and glyphosate – Strategies, lock-ins and diversity in the Belgian Walloon Region

Manon S. Ferdinand, Pierre Bertin, Philippe V. Baret  
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Conservation Agriculture (CA) consists of an alternative agriculture system based on the protection of the soil. CA promotes a minimum tillage and soil disturbance, a permanent soil cover and a diversification of crops [1]. In Belgium only 270 ha are under CA practices [2]. The expansion of the system is limited by dependence to glyphosate [3, 4, 5], a total herbicide that may be banned in 2022 [6]. By conducting semi-directed interviews in Wallonia, this study identified two kinds of strategies to build a CA without glyphosate. The first one includes strategies which optimize glyphosate efficiency (optimal spraying conditions, spray solution composition, low volume technique...) while the second gathers strategies which replace glyphosate for the destruction of cover crops, volunteers from previous cropping and weeds (soil working tools, fodder breaks, pasture...). Farmers use some strategies rather than others based on different trade-offs, which are economic, managerial, institutional, environmental, social or technical. Understanding the interaction between these compromises and the choice of strategies employed allows to define the transition pathways of farmers practicing CA or those who may soon adopt it.

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## **Collaborative design of a sustainability diagnosis based on simple in-field measurements for Walloon field crop systems**

Lola Leveau, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, 1348, Belgium

Hugues Falys, Platform “Fermes universitaires de Louvain”, UCLouvain, Louvain-la-Neuve, 1348, Belgium

Pierre Bertin, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, 1348, Belgium

In Wallonia, different alternative models of agriculture are developing. Conservation agriculture or organic agriculture constitute good examples of that. Each movement is creating its own definition and assessment system for sustainability (Vankeerberghen and Stassart, 2016), which makes communication between farmers and objective comparison of these new farming systems difficult.

In this complex structure, one of the roles of universities is to allow an objective evaluation of the different existing farming systems, based on relevant indicators (Lebacq *et al.*, 2013), and clear sustainability objectives.

During five years, we will develop a diagnosis tool for field crop systems. The indicators employed will be based on the concept of ecosystem services. The in-field measurement methods will be co-designed with a network of Walloon farmers, and will be tested during three growing seasons on their fields. Our research question is the following: can we collaboratively design a diagnosis tool for cropping systems that is, on the same time:

- Generator of scientific knowledge that is exploitable by researchers;
- Appropriable by the farmers and their technical advisors;
- Transferable beyond the initial farmers network;
- Rigorous and in accordance with a clear sustainability definition?

This work presents the objectives and the planning of the thesis.

Lebacq, T., Baret, P., & Stilmant, D. (2013). Sustainability indicators for livestock farming. A review. *Agronomy for Sustainable Development*, 33(2), 311-327.

Vankeerberghen, A., & Stassart, P. M. (2016). The transition to conservation agriculture: an insularization process towards sustainability. *International Journal of Agricultural Sustainability*, 14(4), 392-407.

## Enabling high-throughput phenotyping of dynamic root traits in bread and durum wheat

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Over the last years, high throughput phenotyping has become increasingly popular in plant biology and breeding. This evolution has been enabled by the rapid progress in sensor technologies and is now supported by demonstrations of the scientific value of carefully designed phenomics experiments. Root phenotyping has been progressing at a slower pace, not only because the constraints to root observation are many, but also because root scientists are still not unanimous on how to represent root system morphology. In a quest for parsimonious root representations that capture both spatial and temporal domains, our group has developed a root phenotyping installation that allows us to evaluate root morphology at high spatial and temporal resolution.

We present here a new image and data analysis pipeline that yields information on the dynamics of root growth and production, as well as root angles and diameters. As these variables are the main parameters of mathematical models of root system architecture, they can be used to simulate virtual root systems that allow the prediction of functional variables that are hardly accessible on real plants. The pipeline processes more than 150.000 images in about 12 hours, followed by supervised validation and outlier identification. We also illustrate the pipeline with experiments on durum and bread wheat diversity panels, in the framework of the SolACE European multi-actors project (<http://www.solace-eu.net>).

## ABSTRACT

### Biological activities of some Plant-Associated *Bacillus sp.* and *Paenibacillus sp.* upon growth in root exudates of Maize (*Zea mays*) and Tomato (*Lycopersicon esculentum*) varieties cultivated in Democratic Republic of Congo (DRC)

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Plant-Associated Bacteria (PAB) revealed many potentialities for plant protection against phytopathogens. To protect plant against these enemies, PAB produce bioactives compounds such as Cyclic Lipopeptides (CLPs). These molecules can act via many ways like induced systemic resistance in the host plant and antibiosis. In practice, PAB are applied either in the soil or on the phylloplan.

The aim is to evaluate the ability of PAB to colonize the roots of maize and tomato and to produce various CLPs: surfactins (SF), fengycins (FN), iturins (IT) and fusaricidins (FU).

Wild types strains *Bacillus velezensis* GA1, *B. v.* S499 and *Paenibacillus polymyxa* 56 were tested. Roots exudates (REs) were collected from one variety of maize and three of tomato. The PAB were cultivated in REs collected from those plants. Their growth was evaluated by Optical Density and CPL production was analyzed by using UPLC-MS.

We found that these PAB can grow in the REs of these 2 plants. Growth was better in maize (S499:  $0,091 \pm 0,022$ ; GA1:  $0,071 \pm 0,006$ ; Pp56:  $0,074 \pm 0,002$ ) than in tomato even if low comparatively to the control (LB medium). About CLPs production, all PAB were able to produce interesting biomolecules involved in biocontrol of phytopathogens. SF, FN and IT were produced by GA1 and S499 in maize and tomato exudates. Pp56 produces FU, another kind of CLP with antimicrobial activity and specific for this bacterium.

PAB tested could be used to protect plant against soil-borne pathogens because they can most probably colonize the roots of maize and tomato based on their capacity to use exudates for growth and based on their ability to efficiently form CLPs which represent major compounds involved in the antagonistic activity against fungi as suggested by our bioassays.

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## BIOLOGICAL NITRIFICATION INHIBITORS FOR THE MITIGATION OF N LOSSES IN WHEAT-PRODUCTION SYSTEMS

Izargi Vega-Mas<sup>1</sup>, Ivan Jáuregui<sup>1</sup>, Pierre Delaplace<sup>1</sup>, Hervé Vandeschuren<sup>1</sup>, Cécile Thonar<sup>1</sup>.

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The widespread use of synthetic nitrogen (N) fertilizers, has promoted the productivity and profit in agricultural systems. However, due to the low N use efficiency of crop plants, the intensive use of fertilizers entails the loss of N from the plant-soil system via NO<sub>3</sub><sup>-</sup> leaching and/or N gas emissions produced by N-transforming microbial processes. Interestingly, certain plant species as wheat, brachiaria or Sorghum have shown the capacity to suppress the activities of soil nitrifiers using organic compounds exudated from their roots (Coskun et al. 2017, Subbarao et al. 2017). These compounds (Biological Nitrification Inhibitors, BNIs) delay the oxidation of NH<sub>4</sub><sup>+</sup> into NO<sub>3</sub><sup>-</sup> that occurs during the nitrification, keeping the NH<sub>4</sub><sup>+</sup> in the soil available for the plant for longer periods while reducing, at the same time, the N losses from the soil.

Due to the relevance of wheat for human food and livestock feed in temperate countries, our aim is to develop new innovative strategies for the identification of new compounds from wheat plants as an environmental friendly alternative to the application of synthetic nitrification inhibitors.

On one hand, we tested the production and activity of BNIs in root exudate samples that were obtained from several hydroponically grown wheat genotypes with a bioassay (modified from O'Sullivan et al. 2016 and Sun et al. 2016) and based on the detection of NO<sub>2</sub><sup>-</sup> produced by *in vitro* grown *Nitrosomonas europaea* nitrifying bacteria. On the other hand, the *in situ* effect of BNIs exudated by roots over the soil nitrification (and denitrification) process was characterized in soil grown conditions for two contrasting wheat genotypes in response to soil N nutrition.

Coskun D, Britto DT, Shi W, Kronzucker HJ (2017) Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. *Nat Plants* 3: 17074

O'Sullivan CA, Fillery IRP, Rope MM, Richards RA (2016) Identification of several wheat landraces with biological nitrification inhibition capacity. *Plant Soil*. 404: 61-74.

Subbarao GV, Arango J, Masahiro K, Hooper AM, Yoshihashi T, et al. (2017) Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology. *Plant Sci*. 262: 165–168.

Sun L, Lu Y, Yu F, Kronzucker HJ, Shi W (2016) Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. *New Phytol*. 212: 646–656.

## Oral Presentation

# Unfolding the Evolutionary Modular Structure of The Plant Virus Modulome:

Exploring Proteomics for Applied Biological Predictions in Plant Metaviromics and Disease Epidemiology

Rachid Tahzima<sup>1</sup>, Annelies Haegeman<sup>2</sup>, Kris De Jonghe<sup>2</sup>, Yves Brostaux<sup>1</sup>, Mart Krupovic<sup>3</sup>, Sébastien Massart<sup>1</sup>

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The viruses infecting plants represent very abundant populations, which display a remarkable diversity of virion architectures, genomic structure and replication strategies<sup>1</sup>. An extensive quantitative comparison study of structural protein folds and functional modules (SCOP; CATH; Pfam), coupled with assessment of other genomic and biological features, such as intrinsic protein disorder, virion architectures and vector transmission modes, revealed an unexpectedly limited repertoire of protein domains and functions, here coined the Modulome<sup>2</sup>, associated with the plant virome. We uncovered that the plant virome proteomic make-up is shaped by multiple, evolutionarily independent adaptations, including modular recruitment, duplication and loss of diverse host protein domains. Furthermore, numerous unique or shared hallmark modules (Coat Proteins) could be specifically correlated to virion assembly, taxonomical characters and plant virus/vector determinants of epidemiological significance. Collectively, our results show that application of the modulome approach accurately reconstructs the taxonomic classification of known and newly discovered plant viruses together with better and more accurate assignments. Together with more fundamental evolutionary aspects, this study provides long term applications and further predictive insight into understanding pathogenic and biological properties of plant viruses.

## References:

<sup>1</sup>Koonin EV, Dolja VV, Krupovic M. (2015) Origins and evolution of viruses of eukaryotes: The ultimate modularity. *Virology* 479–480:2-25; PMID:25771806; <http://dx.doi.org/10.1016/j.virol.2015.02.039>.

<sup>2</sup>Tahzima, R., Haegeman, A., De Jonghe, K., Brostaux, Y., and Massart, S. (2018). “Unearthing Planet’s Plant Virus Modulome: Exploring Plant Virus Proteome Modularity for Taxonomic Classification and Biological Predictions”. International Congress of Plant Pathology ICPP2018 / The American Phytopathological Society (APS) - Proteomics / Metabolomics / Genomics. Boston – Massachusetts, USA (Poster – Scientific Communication).

Session 2 (BV)

# Biotechnology

14h00 -15h00

**Chairman: Dieter Vandenheuvel – UAntwerp**

The importance of osmoregulation in ethanol tolerance

Thomas Schalck (KULeuven)

Increased oleochemical production by implementing a rapid microbe optimization platform

Paul Matthay (KULeuven)

Biodegradable plastic, a sustainable solution for the environment?

Alice Delacuvellerie (UMons)

Molecular engineering applied to the directed synthesis of fengycin by *Bacillus subtilis*

Quentin Gras (University of Lille – ULiège)

Flash presentations

## Study of antagonistic activity of associative Rhizobacteria

Rakhilya Aipova, Aizhan Abdikadirova, Askar Kurmanbayev

Laboratory of ecological biotechnology, National Center for Biotechnology, Kazakhstan

In Kazakhstan's agriculture, root rot, brown rust and septoriosiis are extremely dangerous for spring wheat, which occur almost annually depending on the natural conditions of the year and other factors. The main damage to agriculture is caused by phytopathogenic fungi of the genera *Fusarium*, *Alternaria*, *Penicillium*, etc. [1]. The purpose of the study was the selection of effective strains of rhizobacteria of the genus *Bacillus* spp. with antifungal properties against plant pathogens. The objects of study were a laboratory collection of rhizobacteria isolated from various ecosystems of Kazakhstan. The antagonistic activity of the strains was studied by the stroke and well method [2]. Phytopathogenic fungi of the genera *Fusarium* spp. and *Alternaria alternata* were used as test objects. The antagonistic activity of collection strains of rhizobacteria to phytopathogenic micromycetes was studied. It was shown that the level of antagonistic activity of the strains is different. The activity against *Fusarium* spp. and *Alternaria alternata* based on the size of the zone of inhibition of growth of test cultures and the degree of antagonism increases in the series: *Azotobacter chroococcum* 3/1Ac - 15 mm, *Stenotrophomonas rhizophila* 8P - 17 mm, *Rhizobium* spp. 2/1M - 20 mm, *Paenarthrobacter nitroguajacolicus* 1Al - 25 mm, *Bacillus. mojavensis* - 35 mm. Thus, of the studied strains, the most pronounced *B. mojavensis* strain had the most pronounced antifungal effect.

The work was performed as part of the program. Ministry of Agriculture of the Republic of Kazakhstan program BR 06349586.

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- 2 Zvyagintsev D. G. Methods of soil microbiology and biochemistry. - M.: Publishing House of Moscow State University. -1991. - P.59 - 75.

## **CINNAMALDEHYDE MITIGATES PLACENTAL VASCULAR DYSFUNCTION OF GESTATIONAL DIABETIC RATS AND PROTECTS AGAINST THE ASSOCIATED FETAL HYPOXIA: A MECHANISTIC APPROACH**

Hosni A.<sup>1,2</sup>, Abdel-Moneim A.<sup>1</sup>, Hamid Abd-El M.<sup>1</sup>, Adb-El Twab S.<sup>1</sup>, and G. Beemster<sup>2</sup>

<sup>1</sup> University of Antwerp, Belgium

<sup>2</sup> Beni-Suef University, Egypt

Gestational diabetes (GD) is a common pregnancy disorder, coupled with altered placental vascular reactivity and has severe consequences for fetal growth. Our previous findings revealed a potential safe hypoglycemic action of Cinnamaldehyde (Ci) on a rat model of GD [1]. This study aims to unravel the placental responses to GD through transcriptional, biochemical and histopathological analyses and how Ci mitigates these effects in comparison to glyburide/metformin (Gly/Met). We used the fatty-sucroed diet/streptozotocin rat model of GD [2]. Oral doses of Ci or Gly/Met were given daily, one week before mating onwards. On day 20 of pregnancy, maternal and fetal blood was collected for glucose, insulin, erythropoietin (EPO) and hematocrit (Hct) measurements. Placenta was taken for transcriptome, metabolite and enzymatic antioxidant profiling. H&E and PAS-staining were used for histopathological assessments. Fetal liver was excised for iron content evaluation. Fetuses of GD-group showed a significant drop in Hct level, a 4-fold increase in serum EPO and 28% reduction of the liver iron content compared to controls, which indicates fetal hypoxia. Interestingly, both Ci and Gly/Met reduce GD-hyperglycemia but only Ci mitigates fetal hypoxia. Placental transcriptome of GD and Gly/Met-treated groups revealed overrepresented pathways of angiogenesis, hyperoxia and ROS-biosynthesis with evidences of O<sub>2</sub> consumption for glucose oxidation. Biochemical assays confirmed these findings. Additionally, Ci prevents distinct structural alterations of diabetic placenta that mainly include labyrinth hypervascularization and glycogen increment. In conclusion, Ci counteracts GD-induced fetal hypoxia by improving placental function.

## MALTOSE AS CARBON SOURCE USED FOR ANAEROBIC SOIL DISINFESTATION (ASD) INCREASED CROP YIELD AND MODIFIES SOIL MICROORGANISMS IN STRAWBERRY PRODUCTION SYSTEM

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1 Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China

2 Laboratory of Integrated and Urban Phytopathology, University of Liege, Belgium

Because of high toxicity and potential risks for human and environment, the development of alternatives to chloropicrin for soil disinfection on strawberries is a urgent need. Anaerobic soil disinfestation (ASD) used as an organic management practice can effectively control the soil-borne diseases for high-value crops. In this study, we evaluated the ASD during 3 weeks and using maltose at 6 t/ha (Mol6) or 9 t/ha (Mol9) covered with total impermeable film (TIF) to replace Chloropicrin (Cip, 0.375 t/ha) in strawberry production in Changping and Daxing district trial sites in China. The soil treatments also included TIF control (Sol), and untreated and uncovered control (CK).

The results showed that ASD with maltose as a carbon source significantly reduced the colonies of *Fusarium* spp. and *Phytophthora* spp. in the two trial sites compared to CK. The ASD and Cip treatments were significantly reducing the mortality and increased the yield of strawberry in the Changping trial. ASD and Cip treatments increased  $\text{NH}_4^+-\text{N}$  but reduced  $\text{NO}_3^--\text{N}$ , and pH in the soil. The high throughput sequencing of V3-V4 region of 16s rDNA and of the internal transcribed spacer (ITS) region showed that there were no significant differences in OTU richness and evenness of bacterial and fungal community diversity between all treatments. More bacterial and fungal taxa tended to increase rather than decrease in abundances in the ASD treatments, except Mol9 treatment. The strict anaerobic species Clostridiales, Anaerolineales, and Bacteroidales in Mol9 treatment increased by 87%, 456%, and 667% compared with CK, respectively. The analysis suggested that ASD using maltose as a carbon source can be used as an alternative for chloropicrin for pathogen suppression on a strawberry production system.

## Comprehensive Perception Approach of Adoption: Experimenting Hybrid Chinese Maize Varieties in Benin

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### Abstract

The probability of adoption of four Chinese Hybrid Varieties of maize is considered as a favorable perception for these varieties by actors. In order to understand the way of adoption, a panel of actors comprising producers, processors, traders, extension officers, local elected representatives and, above all, end-users, was used as enumerator to evaluate the behavior of those varieties in comparison to the reference maize varieties known as “local” in experiment plots during the vegetative, harvesting and processing phases. For each actor surveyed and for each introduced variety, the comparative index of appreciation (IA) was determined by the difference in perception scores with respect to each of the descriptors evaluated. The adoption of maize varieties within the sites surveyed was affected by the respondent’s social profile (title), the number of varieties already adopted by the respondent, respondent’s experience, age, educational background, membership to an association/organization and the site (research station). The estimation of adoption relative to probabilities (odds ratio) of each variety of maize from the binary logistic regression models revealed only one variety having more than one in two chances for being adopted. Unlike the adoption rate of maize varieties calculated after expensive dissemination efforts, the analysis of probabilities and determinants of adoption somewhat reduces research, pre-extension and extension efforts. The proposed approach allows for a flexible integration of research experiments and field extension concerns of the process of adoption by creating panels of stakeholders around research experiments on research stations.

**Keywords:** adoption probability, adoption rate, maize, China, Benin

### 1. Introduction

The adoption of maize varieties by the stakeholders is a major challenge given the multitude of varieties available (Shiferaw, 2013). However, isn’t it another challenge to prioritize the probability of adoption on the rate or frequency of adoption? If for one, the conventional implementation approach becomes increasingly heavy and very expensive in material, human and financial resources (FAO, 1998), for the other, the “Comprehensive Perception Approach of Adoption” is rather imperceptible. Recall that what is known as agricultural technology transfer is supply driven, while adoption of agricultural technology suggests a demand driven process (Courgeau, 2004). Whether this process is a single stage, or two-stage or multistage decision process is not at stake here (Dimara & Skuras, 2003). This research underscores the process of agricultural technology transfer that it endeavors to abridge at just research stations. Indeed, a good agro-morphological, phyto-pathological and entomological behavior is a bonus for the producer, but it is not decisive in the adoption of an improved maize variety (Dedehouanou et al., 2015, 2017). For instance, a good knowledge of the descriptors whose sensitivity is high with the actors directs very precisely on the research and selection efforts to be made with respect to a given

variety (Aly, 2001; Dewaminou, 2004). Moreover, the relative appreciation of certain descriptors, especially those of the vegetative, harvesting or processing phases, suggests the nature of the complementary research to be carried out: whether it concerns agronomic research in order to highlight the potential (Hononta & Agbetogan, 2002), or whether it is a genetic research for the expression or mitigation of given attributes (seed research) (Dewaminou, 2004).

Perception assessment implemented here leads to the identification of factors and probability of adoption, approach less complex than the traditional agricultural technology transfer (Ntsama Etoundi & Kamgnia, 2008; Dedehouanou et al., 2015). The pending issue is how to establish a link between probability of adoption and adoption rate; which would justify the new approach. Such an achievement leading to spared resources and time would represent a breakthrough in the process of agricultural innovation and transfer in the developing countries. Therefore, this paper endeavors to tentatively link the probability and determinants of adoption with the rate and determinants of adoption, pioneering innovative pathways to a costless and timeless approach to agricultural technology transfer.

## 2. Materials and Methods

### 2.1 Materials and Experiment Zones

Fifty Chinese hybrid varieties were introduced and tested in Benin. Four of those varieties revealed strong attributes with regard to phyto-pathological and pest concerns. They further demonstrated strong spatial and rustic fitness. That is the rationale of those four varieties being experimented and evaluated with respect to their agronomic, disease resistant, pest control and processing performance in three different and important agro-ecological zones of Benin. Experimental plots were installed at three research stations: Niaouli at CRA-Sud (site 1), Gobé at CRA-Center (site 2), and Ina at CRA-North (site 3). Four varieties were then introduced in 2014-2015 agricultural campaign. The first of those varieties was “Guidan 162” (denoted T2 variety, 84-day seed-maturity cycle, potential yield of 7 t/ha, yellow color grain, spike insertion height of 86 cm), described by Affokpon et al. (2015). The second of those varieties was “Jingyuan 688” (denoted T3 variety, seed-maturity cycle of 77 days, potential yield of 3.7 t/ha, yellow color grain, spike insertion height of 45 cm), described by Sikirou et al. (2015). The third one was “Jinyu No.8” (denoted T4 variety, 80-day seedling-maturity cycle, potential yield of 6.5 t/ha, yellow color grain, spike insertion height of 58 cm), described by Dedehouanou et al. (2015). And the fourth one was “Xianyu 335” (denoted T5 variety, 79-day seedling-maturity cycle, potential yield of 4.3 t/ha, yellow color grain, spike insertion height of 49 cm), described by Akissoe et al. (2015). The four Chinese hybrid maize varieties were then ranked according to the decreasing weight performance of their yields as follows: T2, T4, T5 and T3. The test was installed according to the randomized block design with four replicates. As part of the comprehensive perception evaluation, respondents compared the four Chinese varieties with their own local varieties. In reality, local variety was chosen by the respondent and acted as a reference/baseline for evaluation (Dedehouanou et al., 2015, 2017).

### 2.2 Sampling and Data Collection

The perceptions of producers, food processors, maize traders, extension officers, local elected officials and end-users on the agro-morphological, phyto-sanitary, physico-chemical characteristics and the processing capability, packaging and storage capacities of four Chinese hybrid maize varieties were evaluated through semi-structured interviews of individual respondents. Three areas were explored known as the Guinean zone (site 1), the Sudano-Guinean zone (site 2) and the Sudanian zone (site 3). To this end, enumerators were listed according to some specific profile. For instance, gender considerations were at stake in general. So, was membership or not to a farmers’ organization for producers. The criterion of having or not participated in an assessment of maize in the past was also relevant. There were also a few purposeful biases. The first important bias was to select the chief of the village in which the research station is located. This is to smooth relationships between agricultural research stations and villagers in a Research & Development perspective. The second important bias was to select local extension officers as their role in the agricultural technology transfer is to communicate the strengths and qualities of a given technology to producers. This is to obtain their adhesion beforehand so as to prompt their willingness to transfer the new varieties. Overall thirty (30) enumerators were selected by site. The gathering of enumerators in order to investigate agricultural experiments happened three times: (1) ten days after sowing, the lifting phase; (2) at harvest, the harvesting phase; (3) after conservation and during food processing, the processing phase. One important criterion that was set in order to validate perception data was enumerators’ consistency with respect to the three research phases. If an enumerator failed to participate in one phase (maybe he/she was not available, or he/she asked relatives to answer on his/her behalf), his/her data sheet was withdrawn. This suggests a good deal of communication and motivation on both

researchers and participants sides. For instance, there was a serious consideration to count upon researchers among enumerators in the beginning. Because of administrative concerns, all researchers could not be available for all three phases. Researchers were then all withdrawn in the end. Respectively 18, 18 and 19 enumerators were finally surveyed on sites 1, 2 and 3 for all three research phases. It is very illustrative to notify that the specialist of phyto-pathology recommended to slash and burn organs of variety “Jinguyuan 688”/T3 in the Sudano-Guinean zone (site 2), because of disease attacks.

The main topics of the interviews were the perceptions on: i) the lifting phase [on seedling size depending on their age, robustness of seedlings, number of organs attacked on the plant, number of diseases on the plant, number of organs damaged on the plant and number of pests on the plant]; ii) the harvesting phase [size and volume of the spike, number of spikes per plant, dimensions (length, width and thickness) of the grains, drying level of husks, phyto-sanitary state of the spikes at harvest, and attack of the extreme side of the spike at harvest, number of pests per spike at harvest, maize kernel attack on spike at harvest, vitreous nature and friability of grain, grain indentation, structural composition (pericarp, germ and endosperm) of grains and color of grains]; and iii) the processing phase [elasticity of food derivatives/paste organoleptic quality of derivatives/paste elasticity of food derivatives/organoleptic quality of paste “Gambalilifin”/elasticity of paste “Gambalilifin” food/“Akassa” paste, organoleptic quality of derivatives/akassa and Degree of culinary fitness/diversity in number of foods]. Perceptions were reported for Chinese hybrid maize varieties and for the best local varieties. The appreciation scores of each perception variable (descriptor) varied from -2 for the least appreciated variables to +2 for the most appreciated variables (-2, -1, 0, +1 and +2, respectively).

### 2.3 Methods: Statistical Data Processing and Analysis

The socio-economic characteristics of the respondents were first determined by means of a frequency distribution. This characterization was supplemented by a Principal Component Analysis (PCA), based on a matrix with rows, different categories of actors and for columns, variables related to the number of varieties already adopted, membership or not to a farmers' association/organization, participation or not in an assessment on maize crop in the past and the exercise or not of other income generating activities (Dedehouanou et al., 2015). This statistical analysis was performed with the software MINITAB Release 14.

For each actor investigated and for each introduced variety, the index of comparative assessment (ICA) of the variety introduced and the reference variety was determined by the difference in perception scores from the two varieties ( $Score_{[introduced\ variety]} - Score_{[reference\ variety]}$ ) for each of the variables (descriptors) of evaluated perception. For a given variable, the introduced variety is well-liked (compared to the reference variety) if  $ICA > 0$ .

The correlations between various characteristics of the varieties introduced were extracted by a step by step canonical discriminant analysis (stepwise discriminant canonical analysis) on the characteristics relating to lifting, harvesting and culinary/processing phases of the introduced varieties. This analysis was carried out with the STEPDISC procedure under SAS (version 9.2).

The discriminant canonical analysis was followed by a discriminant factorial analysis designed to describe, through a system of canonical axes, the varieties and the sites according to the relevant variables (descriptors) retained by the step by step canonical discriminant analysis. The statistical analysis was carried out, by site and for all sites, using the CANDISC procedure under SAS (version 9.2).

An index of appreciation (Ia) has been defined for given site (s), variety (k) and phase (j) (Hosmer & Lemeshow, 1989),

$$Ia_{ikji} = \frac{1}{n} \sum_{i=1}^n (Score_{[introduced\ variety]} - Score_{[reference\ variety]}) \quad (1)$$

where, i denotes the index variables (descriptors) selected from the step by step canonical discriminant analysis.

This index varies from -4 to +4 and reflects the overall opinion of the respondents on the variety concerned. If,

$Ia_{ikji} < 0$ , the introduced variety is less appreciated than the reference variety of the respondents;

$Ia_{ikji} = 0$ , respondents/actors are indifferent to the two varieties (introduced and reference);

$Ia_{ikji} > 0$ , the introduced variety is more appreciated than the reference variety of the respondents.

An index of overall appreciation (Ig) was determined per respondent, per site and for all sites from the varieties of characteristics put to use in the step by step canonical discriminant analysis (Hosmer & Lemeshow, 1989),

$$Ig = \frac{1}{n} \sum_{i=1}^n (Score_{[introduced\ variety]} - Score_{[reference\ variety]}) \quad (2)$$

where, i denoting the index of the relevant characteristics retained.

A maize variety could probably be adopted if its Ig is greater than zero ( $Ig \geq 0$ ). It was then possible to determine who could adopt the introduced varieties in the future (probable adopter = 1 and probable non adopter = 0).

The relationship between the adoption of a variety by a person and the socio-economic characteristics of the person was then modeled using a binary logistic regression (Hosmer & Lemeshow, 1989):

$$\pi(x) = \text{Prob}(Y = 1/X = x_1, x_2, \dots, x_k) \quad (3)$$

with the dependent variable (in this case, the adoption or not of a variety:  $y = 0/1$ ) and the independent variables (here, socio-economic characteristics:  $x_1, x_2, \dots, x_k$ ).

The probability of adoption of a variety was of the form (Hosmer & Lemeshow, 1989),

$$\text{Log}\left(\frac{\pi(x)}{1-\pi(x)}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k \quad (4)$$

or,

$$\pi(x) = \frac{e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k}}{1 + e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k}} \quad (5)$$

where,  $\beta$  ( $\beta_1, \beta_2, \dots, \beta_k$ ) represents the vector of regression coefficients and  $x$  ( $x_1, x_2, \dots, x_k$ ) the vector of socio-economic characteristics measured by investigation.

This analysis was made for the probable adoption of all varieties without distinction and for the probable adoption of each variety by site and for all sites. It enabled to infer the probability of adoption of the varieties by the average of the individual probability of adoption of the variety by the respondents. This statistical analysis was carried out with software R version 3.0.2.

### 3. Results

#### 3.1 Socio-economic Determinants of the Likelihood of Adoption of Four Chinese Hybrid Maize Varieties

The binary logistic regressions (Table 1) showed that the probability (future decision) of adoption of a maize variety would depend significantly on the maize variety and the site (agro-climatic zone). Moreover, the social profile and the number of years of experience of the enumerator determined the probability of adoption of maize varieties on sites 1 (Guinean zone) and 2 (Sudano-Guinean zone), while participation in an assessment of maize in the past determined the probability of adoption of maize varieties on sites 2 (Sudano-Guinean zone) and 3 (Sudanian zone). Membership to a farmers' association/organization and the level of education of the respondents, respectively, affected the likelihood of adoption of maize varieties on sites 1 (Guinean zone) and 3 (Sudanian zone).

Estimating the relative chances of adoption (odds ratio) of maize varieties derived from binary logistic regression models revealed that T2 variety is 0.08, 0.02 and 0.01 times more likely to be adopted than T4, T5 and T3 varieties, respectively. In addition, maize varieties on site 1 (Guinean zone) are 27.93 and 9.31 times more likely to be adopted than on sites 2 (Sudano-Guinean zone) and 3 (Sudanian zone), respectively. At each site, T2 variety has more chance of being adopted than T3, T4 and T5 varieties, in particular on the site 2 for which T2 variety is 2.03, 0.02 and 4.16 10<sup>-10</sup> times more likely to be adopted than T4, T5 and T3 varieties, respectively. Thus, extension agents are more likely to adopt maize varieties than local elected officials (on sites 1 and 2) and producers (on site 2), while enumerators who do not belong to a farmers' association/organization are more likely to adopt than those who belong to an association/organization on sites 1 and 2. It is the same trends for the uneducated versus educated enumerators on site 3.

The decision to adopt the different varieties of maize within the prospected sites (Table 2) was found to be significantly influenced by the social profile of the respondent (for T2 variety on each site and T4 variety on site 2, Sudanian zone), the number of varieties already adopted by the respondent (for T2 variety on site 1, Guinean zone; and T4 variety on all sites) and the respondent's experience in years (for T5 variety on site 1, Guinean zone; T4 variety on site 2, Sudano-Guinean zone and T5 on all sites). Age, level of education and membership of respondents to a farmers' association/organization and site were critical for T4 variety on site 2, Sudano-Guinean zone and all sites, T2 on Site 3, Sudanian zone and T5 variety on all sites, respectively.

Table 1. Effect of socio-economic characteristics on the overall adoption probability of the four Chinese hybrid maize varieties

Sites	Variables	Degree of freedom	Probability of adoption
Site 1	Maize variety	3	0.000
	Social profile of respondent	1	0.040
	Number of years of experience of respondent	1	0.001
	Membership to an association/organization	1	0.001
Site 2	Maize variety	3	0.002
	Social profile of respondent	2	0.030
	Number of years of experience of respondent	1	0.029
	Participation in an assessment of maize in the past	1	0.000
Site 3	Maize variety	3	0.000
	Educational background of respondent	3	0.001
	Participation in an assessment of maize in the past	1	0.004
Global	Maize variety	3	0.000
	Site	2	0.033

*Note.* Only significant effect variables on the adoption of maize varieties are presented;

The degree of freedom is set for the number of modalities that takes the variable minus 1;

Site 1: Guinean zone, Site 2: Sudano-Guinean zone, Site 3: Sudanian zone.

Table 2. Effect of socio-economic characteristics on the probability of adoption of the four Chinese hybrid maize varieties

Sites	Maize varieties	Socio-economic characteristics	Degree of freedom	Probability of adoption
Site 1	T2	Social profile of respondent	1	0.007
		Number of varieties already adopted	1	0.011
	T5	Number of years of experience	1	0.040
Site 2	T2	Social profile of respondent	2	0.001
		Age of respondent	1	0.015
	T4	Social profile of respondent	2	0.012
		Number of years of experience	1	0.005
		Site	2	0.000
Site 3	T2	Social profile of respondent	2	0.004
		Educational background of respondent	3	0.010
Global	T4	Number of varieties already adopted	1	0.005
		Number of years of experience	1	0.049
	T5	Membership to association/organization	1	0.003
		Site	2	0.000

*Note.* Only significant effect variables on the adoption of maize varieties are presented;

The degree of freedom is set for the number of modalities that takes the variable minus 1;

Site 1: Guinean zone, Site 2: Sudano-Guinean zone, Site 3: Sudanian zone.

The estimation of relative chances of adoption (odds ratio) of each variety of maize per site and for all sites from the binary logistic regression models generally showed that on site 1 and site 3 (Sudano-Guinean zone in particular, and Sudanian zone), T4 variety is more likely to be adopted, whereas non-membership to a farmers' association/organization implies a greater chance of adoption of T5 variety on site 3 (Sudanian zone) than membership to a farmers' association/organization. Moreover, extension agents are more likely to adopt T2 variety than local elected officials on site 1, local officials and producers on site 2, producers and food processors on site 3. They are also more likely to adopt variety T4 than local elected officials and producers on site 2.

### 3.2 Probability of Adoption of Four Chinese Hybrid Maize Varieties

The estimation of the probability of adoption of maize varieties by site and for all sites surveyed is presented in

Table 3. Whatever the site, T2 variety has the highest adoption probabilities (greater than 0.60) while T3 variety is granted the lowest (less than 0.06). Moreover, T4 variety has the highest probability of adoption (0.50) on site 2. The same trends are observed across all surveyed sites (Probabilities of adoption of T2 and T3 varieties: 0.5455 and 0.0182, respectively).

Table 3. Probability of adoption of maize varieties by site and for all sites

Sites	Maize varieties	Probability of adoption	Sites	Maize varieties	Probability of adoption
Site 1	<b>T2</b>	<b>0.667</b>	Site 3	<b>T2</b>	<b>0.632</b>
	T3	0.000		T3	0.000
	T4	0.000		T4	0.053
	T5	0.056		T5	0.053
Site 2	T2	0.444	Global (all 3 sites)	<b>T2</b>	<b>0.546</b>
	T3	0.056		T3	0.018
	<b>T4</b>	<b>0.500</b>		T4	0.146
	T5	0.111		T5	0.055

Note. Site 1: Guinean zone, Site 2: Sudano-Guinean zone, Site 3: Sudanian zone.

#### 4. Discussion

The following two assertions should be used with discernment. These are the probability or likelihood of adoption and adoption rates or frequencies. The same holds true for their determinants, even if certain socio-economic characteristics remain neutral as much with respect to objective as well as subjective probabilities.

##### 4.1 Probabilities and Determinants of Adoption Versus Rates and Determinants of Adoption of Maize Varieties

The results presented with regard to the probability/likelihood and the determinants are distinct from the adoption rate and the determinants of adoption found in the literature. For instance, an adoption rate of 10% (Ntsama Etoundi & Kamgnia, 2008) appears to be very far behind the likelihood of adoption of 0.50 (probability of adoption) obtained by the best Chinese hybrid maize variety at all three sites in Benin. The decision process described in the present research is rather the modeling of the revealed preferences for descriptors of introduced maize varieties, as opposed to the two-stage adoption decision process (Dimara & Skuras, 2003). By integrating the multiple constraints acting on the adopting actor, it is instructive to infer that at the phase of adoption in the fields of agricultural producers the rate of adoption would be very much reduced (Dedehouanou et al., 2015, 2017). However, actors' gender did not affect the rate of adoption as in the present case study, although consistently, Ntsama Etoundi and Pedelahore (2010) emphasized maize variety, socio-economic profile, number of years of experience and participation in an assessment on maize in the past on one side, number of varieties already adopted and age of enumerator on the other side as determinants of the adoption rates. Those findings are also established in the present case study. As expected, admitting the principle of calculating the probability, the principle of sufficient reason leading to its separation from the principle of causality between one and the other variable, is relevant to elucidate the tendency at hand (Jorland, 1993). The challenge here would stem from establishing equivalence between probability of adoption and rate of adoption, i.e. adoption performed from agricultural research stations being equivalent to the conventional mode of adoption of agricultural technology, although both processes have not benefited from the same circumstances or determinants in the outset. Therefore, predictions of adoption are likely at the research station if necessary provision is safeguarded by stakeholders of the agricultural technology transfer process. However, this is very suggestive unless authors on both sides, research stations with a comprehensive approach of adoption and real productive systems with the conventional approach of agricultural extension, reach consistent results for decision making and interventions.

##### 4.2 Probability and Determinants of Adoption: Towards the Rate and Determinants of Adoption of Maize Varieties

On the basis of the differences between the probability/likelihood of adoption (subjective probability) and the rate or frequency of adoption (objective probability) (Courceau, 2004), the two results cannot be equivalent. Unlike Affokpon et al. (2013), who highlighted the absence or presence of previous crop effects on maize crop yields, these results suggested to forgo such circumstances. This is not consistent with Da-Gbadji, Dedehouanou, Hounnandan, Zoundji, and Kpanou (2019), who constantly emphasized social and economic acceptability in

the adoption of agricultural innovative technologies. Obviously, various circumstances are to be handled by agricultural producers before their effective move to adoption. Adegbola, Arouna, and Ahoyo (2011a, 2011b) on the one side, and Agbaka, Tano, Borgemeister, Foua-Bi, and Markham (2005) on the other side, respectively substantiated the acceptability arguments such as storage losses of maize due to the devastating pest, *Prostephanus Truncatus Horn*. It is then very relevant to note that in the ex-ante situation, enumerators could not probably pass by the multiple constraints of production services, storage and marketing of grains as for acting agricultural producers (Adegbola, Arouna, & Houedjissin, 2011b). However, various factors and circumstances could streamline the gap between probability/likelihood and rate/frequency of adoption. For example, one important favorable factor for adoption could be the market orientation of maize varieties (Ntsama Etoundi & Pedelamore, 2010; Ntsama Etoundi & Kamgnia, 2008), as the ex-ante evaluation demonstrated in two major producing zones of Benin, Sudano-Guinean zone and Sudanian zone (Dedehouanou et al., 2015; Dedehouanou et al., 2017). But still, it could not justify any kind of generalization. Another important factor in favor of an alignment of both probability and rate of adoption could be the matching of determinants. Indeed, the present findings are consistent with Ntsama Etoundi and Kamgnia's (2008), who also claimed that the determinants such as area, educational level, and membership to a farmers' organization positively affect the adoption rate of improved maize varieties, prompting to "similar causes reach similar effects". A further factor or circumstance in favor of relating probability and rate of adoption could be time length that is needed to consolidate research works on the probability of adoption. This is a missing tail of the present research, experimental observations having taken place only for one annual campaign.

Although the probability and determinants of adoption of maize varieties were obtained from the ex-ante perception evaluation of the characteristics relating to the vegetative, harvesting, processing phases of maize resulting from the experiments, the results could reveal the ex-post evaluation of the adoption rates of maize by actors. Recall that this finding was tentatively inferred elsewhere (Dedehouanou et al., 2015, 2017). It then appears that calculation of probability/likelihood of adoption could lead to tentatively predict the rate/frequency of adoption, bringing about striking possibilities such as shortening the diffusion process, saving scarce resources and catching up with times.

## 5. Conclusion

The expensive technology diffusion approach, which suggests a phase of research station experiments, a pre-extension phase and another phase of technology popularization, could be overshadowed in order to shorten the process. However, the innovative approach only leads to the determination of subjective probabilities of adoption of maize varieties. Nevertheless, with a closer view, many constraints could mark the adoption of varieties with a view to determining objective probabilities of adoption. These constraints are, in this case, regarding the production services, storage and marketing of the grains. The challenge, in no doubt, would be to establish tangible links between the perceptions of stakeholders at research stations and those in producers' fields. It would then be possible, from these interrelationships between probability of adoption and adoption rate, to initiate a new approach to agricultural technology transfer.

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## High throughput microfluidic droplet and particle production

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In the last two decades, microfluidics has been used to produce highly uniform micro-droplets and particles, not achievable with conventional macroscopic techniques. This precise control generated a growing interest within several industries, including pharmaceutical, food and chemical companies. However, the low production rate of microfluidic devices remains one of the main bottlenecks for their use in industrial applications. Here, we experimentally investigate the production of monodisperse microdroplets in new microfluidic devices wherein we increased the number of droplet generators. Indeed, by increasing the flow rates, the flow transits from droplet generation to the unstable jetting regime [1]. Thus, the only way to increase the production is by increasing the number of droplets generators. We propose two different microfluidic devices which have two inlets and several outlets. Co-flow junctions have been used in the first device and T junctions in the second one. We made 30 $\mu$ m PLGA particles by making droplets of dichloromethane+5% PLGA in water with at least a 5 times higher throughput than a classic device with single co-flow junction. We also varied the PLGA concentration to tune the particle size. Additionally, we made hexane droplets in water. We characterized in detail the size and the frequency of the droplets generated per junction. In this study, we show the possibility of increasing the throughput of the generated droplets/particles by increasing the junctions within a device.

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The influence of shear on protein crystallization under constant shear conditions.

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A novel microfluidic device that subjects a solution to a constant shear flow was developed. By taking advantage of the linear velocity profile in a lid driven flow configuration, small volumes ( $10^{-5}$  L) can be subjected to a constant shear profile with a shear rate between 0.1 and  $100\text{ s}^{-1}$  at accurately controlled temperatures between  $20\text{ }^{\circ}\text{C}$  and  $50\text{ }^{\circ}\text{C}$ .

Both theory [1], [2] and experiments [3], [4] have indicated that shear flow affects the nucleation rate, the size of the emerging phase and polymorphism. Not only crystallization (proteins, Active Pharmaceutical Ingredients...) is affected by shear flow, but protein aggregation and fibril formation are other examples of biologically and medically relevant phase transitions enhanced by shear flow [5][6].

The tunable shear can be maintained for extensive and fully controlled times. A dedicated microscope setup for visualization enables the on-chip detection of micron-sized crystals, particles and aggregates. The influence of shear on the crystallization process of the reference protein lysozyme was studied. The results indicate that shear rates between 1 and  $10\text{ s}^{-1}$  decrease solubility and promote nucleation not only in the supersaturated and metastable zones of the phase diagram, but also in the undersaturated zone. A monotonically increasing nucleation rate was observed for shear rates between 1 and  $10\text{ s}^{-1}$ . It is anticipated that the presented methodology can shed light on a variety of phase transitions that are influenced by flow.

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## **Optimizing drone raising in Belgium, first breeding season results and first result of cryopreservation.**

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Honeybee semen conservation is of worldwide scientific interest with increasing urgency to improve results in order to conserve biodiversity. Gamete cryopreservation from selected lines is also of growing interest. Indeed, selection of bees tolerant to varroa is one of the main current objectives in honeybee research. Cryobanking of these gametes will be exploitable internationally by simple transport. In order to improve cryopreservation, different parameters are studied such as influence of drone age. To study this criterion, we need a method to raise large numbers of drones of known age without impairing the colony's stability. We also need objective characteristics of semen quality. One of these is semen viability studied by epifluorescence or flow cytometry using SYBR-14 and propidium iodide dyes. This report describes our controlled caged raising method, its comparison with uncaged methods and first attempts of cryopreservation. It also details the first steps of validation of the fluorescent supravital staining by flow cytometry as a reference to test new dyes. We can conclude that the rearing method allows to raise large numbers of drones of known age and needs to be tested again during the next breeding season and that the flow cytometry technique needs to be further validated with comparison to epifluorescence microscopy.

## Topically applied live lactobacilli for symptom improvement and microbiome modulation in skin conditions

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Inflammatory skin conditions such as acne and atopic dermatitis (AD) affect an increasing number of children and adults worldwide. Their etiology is complex and in addition to various genetic, immunological and environmental factors, patients demonstrate an altered skin microbiome. A reduction in the microbial diversity is often observed and flares are characterized by an abundance of skin pathobionts such as *Staphylococcus aureus* and *Cutibacterium acnes*. Topical probiotics could have a positive effect and function by inhibiting pathogens, restoring the skin microbiome and/or barrier function and executing anti-inflammatory effects on the skin cells.

In an ongoing collaboration between the UA and biotech company YUN, the potential of probiotics for inflammatory skin conditions is being studied. YUN developed a revolutionizing technique to formulate creams containing live bacteria. A proof-of-concept study with topically applied live lactobacilli in patients with mild-to-moderate acne has already shown promising results according to symptom improvement and skin microbiome modulation (BioRxiv). The clinical effectiveness has been confirmed in a placebo-controlled study, which showed a significant reduction of inflammatory lesions by the live bacteria. To correlate these data with microbiome modulation, analysis of all microbial samples, taken during the entire duration of the study, is going on using next generation sequencing. This research is now extended towards other skin conditions. Previous research implicates that topical lactobacilli are promising to answer the need for new and effective therapies for AD. Herefore, more in depth exploration of the working mechanism, biomarkers and skin microbiome is crucial.

## ABSTRACT

**Cassava seed system, from field to laboratory: farmers' practices, knowledge of biotic constraints and molecular characterization of ipomoviruses associated with cassava brown streak disease.**

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Cassava (*Manihot esculenta* Crantz) is a vital crop in Rwanda where it ranks as the 3<sup>rd</sup> most consumed crop. However, its productivity is hampered by several constraints the major one being cassava brown streak disease (CBSD).

The aim was to assess farmers' practices and their knowledge of the biotic constraints to cassava production and molecular characterization of potyviruses associated with CBSD in Rwanda.

A cross sectional study was carried out from May to September 2019 in 13 districts of Rwanda. A total of 390 farmers participated and 130 cassava fields were visited. Statistical analysis was performed with IBM SPSS.

The highest mean field incidence was recorded in Gisagara district (18%). RT-PCR of 130 samples confirmed the presence of cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV). UCBSV was predominant (24.6%) whereas CBSV was only detected in one district (1.5%). Among the 390 farmers selected for the survey, (57.7%) were individual farmers and (42.3%) were in cooperative. Farmers confirmed that cassava is a cash crop (77%) and most farmers use seeds from their own farm (72.3%). Individual farmers, limited access to extension services, use of uncertified seeds, proximity to border and lack of knowledge on disease transmission were found to be associated with the increase of CBSVs infection.

CBSD was detected in all cassava producing districts. Because most cassava varieties cropped in Rwanda are susceptible to CBSD, the incidence of CBSD is expected to increase. Enhancing CBSD awareness, providing clean planting materials and strengthen breeding programs could be used to mitigate the CBSD incidence. Further study on the CBSVs genetic diversity is also required to develop CBSD resistant varieties.

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## The importance of osmoregulation in ethanol tolerance

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Fundamental knowledge about ethanol tolerance development could provide industry-relevant guidelines to improve the bioethanol productivity of fermentative microorganisms. More specifically, increasing tolerance to ethanol could minimize the adverse effects of this alcohol on the producing strain. Here, our research is focused on dedicated *Escherichia coli* osmoregulation systems to demonstrate their relevance in improving tolerance towards lethal ethanol stress. Particularly, adaptive mutations in these cellular pathways heavily influence molecular behavior of these signaling cascades. As a result, cells harboring the EnvZ mutations display a different expression pattern of downstream-regulated stress-response genes, compared to wild-type *E. coli* cells. Ultimately, this shift protects the cell against ethanol toxicity and significantly improves survival in high ethanol concentrations.

## Reducing Taylor-dispersion by AC-electroosmotic mixing for the improvement of chromatography.

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Liquid chromatography is the most used analytical technique within life sciences.<sup>1</sup> Although abundant improvements have been made since the introduction in the year 1900, different fields, like for example proteomics are still in need for further improvement of chromatographic methods.<sup>2</sup> The amount of compounds which could be separated with a chromatography column is based on the dispersion a plug is subject to, when eluted through a column. The amount of dispersion per length of column can be expressed as the plate height, the theoretical value of which is expressed with the van-Deemter equation<sup>3</sup>, which states:

$$H = g(k') + \frac{2D}{u} + u \frac{l^2 c}{D}.$$

Here  $H$  is the plate height of the column,  $g(k')$  is a velocity-independent term due to eddy diffusion,  $\frac{2D}{u}$ , is the term due to axial diffusion and,  $u \frac{l^2 c}{D}$ , is related to the amount of Taylor dispersion<sup>4</sup>.

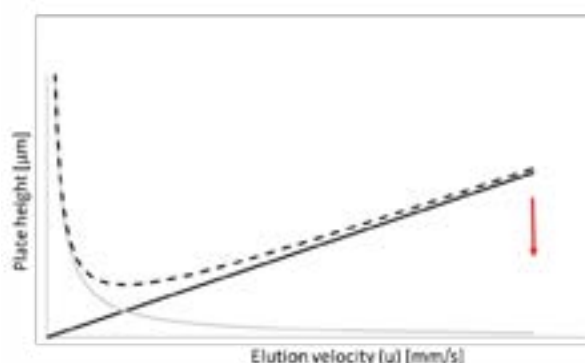


Figure 1- Van Deemter curve (dotted line), with the contribution due to axial diffusion (grey) and the resistance to mass transfer (black), aim of the project is to lower the contribution due to the resistance to mass transfer.

In earlier studies, ordered silicon-wafer based columns were used to reduce the eddy diffusion term to negligible values.<sup>5</sup> Aim of the present project is to reduce the amount of Taylor dispersion by lateral mixing. For this purpose, a silicon chip was constructed with a microfluidic channel in which AC-electroosmotic mixing could be performed. A reduction of the resistance in mass transfer term of around an order of a magnitude was obtained with a device we recently validated. This new method has the possibility to improve the theoretical limits of liquid chromatography in future work.

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## Micropropagation of selected Almond genotypes (*Prunus dulcis* Mill.) cultivated in Eastern Morocco based on their pomological studies

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This study aims to investigate the *in vitro* regeneration ability of selected *Prunus dulcis* genotypes cultivated in four sites from eastern Morocco (Sidi Bouhriya, Rislane, Labsara, and Ain sfa). The almond populations studied are derived from seedling, the trees of these plantations are very heterogeneous in terms of vigor and flowering time. The identification, comparison, and genotype selection were performed based on the pomological traits by using almond descriptor according to the methods described by Gülcan (1985). Parameters with high discriminating values were those related to the shelling, the thickness, the kernels weight, and the double kernels. Genotypes singled out as the most promising for breeding and commercial growing were multiplied by in vitro tissue culture techniques in Murashige and Skoog (1962) (MS) medium containing 30 g.l<sup>-1</sup> sucrose and 7 g.l<sup>-1</sup> agar. Internode sections and leaves of the selected genotypes were used as explants, they were dissected and incubated in varied plant growth-regulator conditions. Of the auxins tested, ct-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) induced adventitious shoots from most of the studied genotypes. For the cytokinins, shoot development was effective in the presence of either 6-Benzylaminopurine (BAP) or thidiazuron (TDZ). The highest callus development was obtained on MS medium supplemented with 2mg.l<sup>-1</sup> of TDZ and 2 mg.l<sup>-1</sup> IBA. All the results obtained showed that the determination of the appropriate type and concentration of phytohormones to have a successful proliferation phase of various *Prunus dulcis* explants depends on the genotype.

**Keywords:** *Prunus dulcis*, Genotypes, Internode sections, Leaves, Plant growth-regulator.

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**Boosting the production of innovative glycolipids by applying integrated omics analyses.**

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Bio-degradable and bio-based surface-active agents are renewable and environmentally friendly alternatives to petroleum derived and oleochemical surfactants. A novel example of biologically produced biosurfactants are bolaform sophorolipids (Bola SL) produced by *Starmerella bombicola*, these molecules can be applied in cosmetics, pharma, nanotechnology,... Recent research demonstrated that there is a market interest for bola SL but production cost is currently too high to be economically sustainable and the productivity has the largest impact on the production cost [1]. Though process development already made some improvements, strain improvement is absolutely necessary. However, this development needs more fundamental in-depth information about the biosynthesis of these molecules by *Starmerella bombicola* and its regulation. A comparative multi omics approach is used to unravel the effects of certain genes and compounds that have an impact on the productivity. Genome and transcriptomic analysis will be conducted while the metabolome will be unravelled with the aid of untargeted and semi-targeted metabolomics. A regulatory pathway model will be constructed out of the multi omics data. This knowledge breakthrough will allow the fine-tuning of SL producing strains. As proof-of-concept, a bola SL producing strain will be engineered to obtain a 4-fold productivity increase.

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## **Sanitizing the international banana germplasm collection for a safe exchange of banana plants around the world**

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Banana is a perennial herbaceous plant and is important for food security, feeding millions of small holders, and as a cash crop in many developing countries. Preserving the biodiversity of *Musa* species is a key issue to address current and future challenges caused by abiotic or biotic stresses, among which the viral pathogens. The most comprehensive repository of *Musa* genetic resources in the world is managed by Bioversity International at the KULeuven, Belgium. These resources are distributed around the world and it is therefore of utmost importance to guarantee the clean health status of the plants. Some viral pathogens, like banana bunchy top virus, banana mild mosaic virus or banana streak virus have an important impact on the banana production. Therefore, the indexing and sanitation program established at the Germplasm Health Unit (GHU) at GxABT (ULiege), is screening and treating the plants from the Genebank collection held by Bioversity International. It consists of detecting the presence of infecting viruses in the plant material, and to further perform virus-cleaning therapies to eradicate the virus(es), putting back viral-free banana accessions for a safe and sustained worldwide distribution. More than 260 banana accessions entered the therapy programs, 52% of them were already send back virus-free to the Genebank, and 31% are ongoing. The protocols used, their performance as well as their adaptation to the genetic diversity of *Musa* will be presented and discussed.

## Potential of URT probiotics against chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is a common upper respiratory tract (URT) disease. While the role of microorganisms in the pathophysiology of CRS is controversial, antibiotics are commonly prescribed. This can promote imbalance of the microbiota and the formation of antibiotic resistant bacteria, so there is a need for alternative treatments. Probiotic bacteria could be such an alternative, by inhibiting pathogens, restoring the microbiome and/or epithelial barrier function, and executing anti-inflammatory effects on the airway epithelium.

Previously, a *Lactobacillus casei* AMBR2 was isolated from a healthy human URT. *L. casei* AMBR2 showed anti-pathogenic and anti-inflammatory effects in antimicrobial and cell culture assays focusing on three important CRS-related pathogens, i.e. *Staphylococcus aureus*, *Haemophilus influenzae* and *Moraxella catarrhalis*. As *L. casei* AMBR2 showed promising probiotic features, it was administered live in healthy volunteers via a nasal spray for two weeks. This was found to be safe and overall well-tolerated. Importantly, *L. casei* AMBR2 was able to temporarily colonize the nasopharynx, despite nasal clearance.

In the future, we aim to test *L. casei* AMBR2 in CRS patients, since topical treatment of patients with niche-specific probiotic isolates could be the most effective (Martens *et al.*, 2018). In addition, more in-depth research regarding the working mechanism of *L. casei* AMBR2 and the link between the URT and lower respiratory tract microbiome is being conducted.

Martens, K., Pugin, B., De Boeck, I., Spacova, I., Steelant, B., Seys, S. F., Lebeer, S., & Hellings, P. W. (2018). Probiotics for the airways: Potential to improve epithelial and immune homeostasis. *Allergy*, (May): 1–10.

## **Increased oleochemical production by implementing a rapid microbe optimization platform**

Paul Matthay, Natalie Verstraeten, Jan Michiels  
Katholieke Universiteit Leuven, Belgium

Fatty acids and other Oleochemicals represent a sustainable and imperishable alternative to the raising demand of petrochemicals. Nonetheless, the current oleochemical production by distillation of plant extracts cannot satisfy the increasing demand in a sustainable way. Recent advances in metabolic engineering arouse bacteria as an alternative, large-scale sustainable way of production. However, commonly applied strain optimization strategies (based on selection of the fittest) cannot be used, since an increased biochemical production is mostly unbeneficial. Hence, we propose a revolutionary rapid microbes optimization platform for single cell selection:

Target enzymes with a known effect on the production of fatty acids will be selected and every codon of production genes will be replaced by all possible alternatives using state-of-the-art genome editing methods, resulting in large mutant libraries. Once constructed, the libraries will be screened using a microfluidics setup. The cells will be encapsulated singly in picodroplets with a sensitive, fluorescent-based detection kit for free fatty acids. Separation of cells removes competition between individual cells and results in selection solely on fatty acid production and not growth, allowing to better maintain the diversity of mutations. In a final step, mutations, that increase fatty acid yield will be identified and combined to create an optimized biocatalyst strain.

## Biodegradable plastic, a sustainable solution for the environment?

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Actually, the main solution for the plastic environmental pollution is the utilisation of biodegradable plastics in place of the non-biodegradable one. The degradation of these polymers is mainly tested into compost condition (70 °C, 90% of degradation in CO<sub>2</sub> after 180 days), but what about the marine environment? Bacterial communities able to form biofilm on plastics and degrade them are unknown. The aim of this study is to better understand the interaction of microorganisms with biodegradable plastics and also verify if these polymers are degradable into natural marine conditions, in the Mediterranean Sea (Corsica). Non-biodegradable (PET, LDPE, PS and PVC) and biodegradable polymers (semi-crystalline PLA, amorphous PLA and PBAT) were submerged during 1 month into the sea: (1) on the sediment (8 m depth) and (2) into the water column (4.5 m depth). Chemical analysis (DSC, GPC, ATR-FTIR) were performed on sampled plastics to observe the potential degradation and the bacterial communities present on plastic-biofilm were studied.

## **Phyllosphere bacteria and air pollutants: potential for bioremediation and impact on the human immune response in vitro.**

Max Dekeukeleire<sup>1</sup>, Wenke Smets<sup>2</sup>, Sarah Lebeer<sup>1</sup>

<sup>1</sup>*University of Antwerp, Belgium*, <sup>2</sup>*University of California*

Air pollution is linked to a plethora of human health problems, and novel strategies to improve air quality are indispensable. Biological removal of pollutants has recently become attractive due to low investment and operational costs, and being eco-friendly.

In this project, we investigated the ability of aerial plant surface (phyllosphere)-colonizing bacteria to bioremediate air pollution and influence immune responses in vitro.

Phyllosphere bacterial isolates were co-incubated with air pollutants (benzene, toluene, ethylbenzene, and xylene (BTEX)) inside the "Tolbox", which is an airtight chamber connected to a gas chromatography read-out. The impact of phyllosphere bacteria in combination with urban pollutants (diesel particulate matter (DEP)) on human immune responses was evaluated in NF- $\kappa$ B-SEAP Reporter Monocytes (THP1-Dual cells).

Phyllosphere bacteria induced variable levels of NF- $\kappa$ B production in THP1-Dual cells depending on the bacterial isolate, and this was further influenced by DEP. After addition of DEP to strong NF- $\kappa$ B-inducing bacteria, a relatively lower NF- $\kappa$ B production in THP1-Dual cells was observed, while the addition of DEP to weak NF- $\kappa$ B-inducing bacteria resulted in a relatively higher NF- $\kappa$ B production. Up to now, none of the tested 17 bacterial isolates were able to bioremediate BTEX and more isolates and pollutants will be tested in the future.

Our results demonstrate that phyllosphere bacteria and pollutants can potentially influence each other's effects on human immune responses. Additional research is being conducted to develop new bioremediation applications and to understand the role of phyllosphere bacteria in human health in relation to air pollution.

### Identification and management of vanilla diseases in Madagascar.

Patrick A. Raveloson<sup>1</sup>, Mondher El Jaziri<sup>2</sup>, Caroline De Clerck<sup>1</sup>, Haissam Jijakli<sup>1</sup>

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<sup>2</sup>Université Libre de Bruxelles, Belgium

About 97% of the real vanilla come from Madagascar worldwide. Madagascar's vanilla is well known for its quality, and its production is very important for the country's economy. However this culture is threatened by the emergence of fungal and viral diseases that have been recorded for a decade, - causing significant devaluations of the production's level of quality and an important reduction of the quantities annually exported.

In order to be able to scientifically construct a strategy of prevention and control against the main pathogens of vanilla, large sampling campaign was carried out in Madagascar's SAVA region. Lianas showing symptoms of cryptogamic diseases such as rot, wilt, necrosis or melanosis were harvested. 75 bacteria and 61 fungi were isolated on NA and PDA medium and identified. Morphological and molecular identifications (based on the sequencing of 16S rRNA and ITS regions) of these isolates have shown that the genus *Fusarium* is the most present (27% of fungal isolates) and is represented by species of *F. oxysporum*, *F. proliferatum*, *F. concentricum*, *F. equiseti*, *F. pseudocircinatum* and *F. mangiferae*. While for bacteria, the genus *Bacillus* predominates (44% of purified bacterial strains).

Many bacteria of the genus *Bacillus* are known for their biocontrol properties; this is why we have tested the potential antagonistic effect of all vanilla-isolated *Bacillus* strains against two species of *Fusarium*. Nine *Bacillus* strains were identified for reducing the growth of the fungi, and are of great potential for the development of a biocontrol strategy against *Fusarium* attacking vanilla in Madagascar.

## **Isolation and characterisation of an isobutyrate-producing methylotroph *Clostridium luticellarii*.**

Camille Petrognani, Ramon Ganigué, Nico Boon  
Ghent University, Belgium

Carbon dioxide is the most abundant greenhouse gas on earth. One of the strategies envisioned to reduce GHG emissions involves capturing and utilising CO<sub>2</sub> for the production of economically valuable products. The possible CO<sub>2</sub> conversion routes include the production of methanol a versatile platform chemical and renewable fuel [1]. Methanol can be subsequently converted into higher value-added chemicals such as carboxylic acids through microbial technologies. Recently, the continuous production of isobutyric acid (iC<sub>4</sub>) has been reported in methanol-fermenting mixed cultures [2]. Developing an iC<sub>4</sub> production route from CO<sub>2</sub> could provide the chemical industry with a sustainable supply of renewable and biobased iC<sub>4</sub>. However, to achieve this goal it is essential to gain further understanding on the organism(s) producing iC<sub>4</sub>, the metabolic pathway used and its metabolic triggers. In this study, we reported the isolation and identification of *Clostridium luticellarii* as the organism responsible for iC<sub>4</sub> production. We also studied the substrate range of *C.luticellarii* as well as the effect of pH on iC<sub>4</sub> production.

## Optimization of biosurfactant production in a trickle-bed biofilm reactor with genetically improved bacteria

Hannah Brück<sup>1,2</sup>, François Coutte<sup>2</sup>, Frank Delvigne<sup>1</sup>, Pascal Dhulster<sup>2</sup>, Philippe Jacques<sup>1,2</sup>

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<sup>2</sup>Univ. Lille, INRA, ISA, Univ. Artois, Univ. Littoral Côte d'Opale, EA 7394 - ICV - Institut Charles Viollette, F-59000 Lille, France

The Gram-positive soil bacterium *Bacillus subtilis* is a potential producer of the very powerful biosurfactant surfactin [1]. In the phytosanitary field, surfactin gains increasing attention for the use as biocontrol agent since it is able to induce systemic resistance in plants [1]. Furthermore, surfactin has many applications in different industrial sectors due to the exceptional foaming and emulsifying properties [1].

Biofilm bioreactors are efficient alternative production systems for bacterial biosurfactants since excessive foam formation can be avoided while a high air/liquid mass transfer can be obtained [2-5]. The widely used *B. subtilis* 168 strain has very low cell adhesion capacities and thus a reduced growth in biofilm-based systems. In this work, genetically modified *B. subtilis* 168 mutants have been designed to improve the natural cell immobilization via biofilm formation. The mutants were first cultivated in a drip-flow biofilm reactor to assess their biofilm formation capacities [6]. Then, the strains with the best performances were selected for the cultivation in a trickle-bed biofilm reactor. The mutants with functional biofilm matrix production showed a significantly improved adhesion capacity compared to the control strain as well as an improved surfactin productivity. The introduced cell filamentation seemed to improve cell cohesion and to decrease cell detachment.

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## **The identification of a new isolate of Banana mild mosaic virus by high throughput sequencing triggers the development of a new diagnostic primer**

**Marwa Hanafi<sup>1</sup>, Wei Rong<sup>1</sup>, Kathleen S Crew<sup>2</sup>, Nicolas Roux<sup>3</sup>, John E Thomas<sup>4</sup> and Sébastien Massart<sup>1</sup>**

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The Bioversity International *Musa* Germplasm Transit Centre (ITC) hosts more than 1,500 accessions covering the genetic diversity of the genus *Musa*. Its objective is to conserve this diversity and supply plant materials to users worldwide. These accessions can be distributed only if virus infection is not detected during indexing. The indexing is carried out by targeted IC-(RT)-PCR for 10 viruses, electron microscopy and symptom observation (De Clerck et al., 2017). One accession (ITC 0763) tested negative by IC-RT-PCR while viral particles were observed by electron microscopy, suggesting the infection by a new virus species or by a divergent viral strain from an existing species.

The accession was sequenced as follows: after RNA extraction, the sequencing library was prepared using the Ribo-Zero™ Plant Leaf Kit (Illumina) for ribodepletion followed by the TruSeq Stranded Total RNA Library Prep Kit (Illumina). The sample was sequenced on the Illumina Nextseq 500 platform (Liège University) and a total of 8,683,460 paired reads (2x150 nt) were obtained. Bioinformatics analysis was carried out using Geneious v9.1 software. The nearly complete genome sequence of a banana mild mosaic virus (BanMMV) isolate was identified.

The primers used for the detection of BanMMV during indexing at Gembloux Agro-Bio Tech are Poty1 and BanMMCP2 (De Clerck et al., 2017).

A careful analysis of the genome sequences complementary to the detection primers revealed four mismatches with BanMMCP2 primer. These mismatches could be the origin of the negative PCR results obtained. To confirm this hypothesis, a new primer was designed at the same genome location as BanMMCP2 but with two additional degenerate bases. Combined with Poty1, the new primer was able to detect the infection by BanMMV. Currently, a retrospective analysis is being carried out on all the banana accessions already indexed to validate the new degenerate primer for use in routine indexing.

## The use of drug loaded microparticles as an aid for beta cell therapy

Jordy Stichelmans<sup>1</sup>, Bharani Sekar<sup>1</sup>, Iwona Ziemecka<sup>2</sup>, Wim De Malsche<sup>2</sup> and Karine Hellemans<sup>1, 2</sup>

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One of the promising therapies for Type 1 diabetes involves the transplantation of insulin producing  $\beta$ -cells, derived from donor cells, or human stem cells in macro-encapsulated devices. But this treatment hasn't reached its maximum potential due to the shortage of donor cells, immune responses of the host, the (lack of) vascularization at the implant site and the micro-environment of the implant site. All these factors should contribute to support the survival of the transplanted cells, but instead a gradual reduction of the number of functional cells is observed after transplantation.

Previous work by our team has shown that glucocorticoids, next to their well-known anti-inflammatory effect, also provide a proliferative signal to cultured  $\beta$ -cells [1]. We therefore hypothesize that a local and sustained release of glucocorticoids at the implant site could protect the implanted cells against inflammation and may as well stimulate their proliferation and survival. We are currently evaluating the use PLGA microspheres loaded with glucocorticoids for this purpose. The solvent evaporation method, which yields polydisperse spheres (1-20 $\mu$ m), is compared to the use of microfluidics to produce monodisperse spheres (30 $\mu$ m). Our preliminary results compare the release kinetics of these spheres and show good tolerability, absence of cytotoxicity and a measurable increase of the number of  $\beta$ -cells within 9 days of culture.

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## Unexpected cellulase activity in the understudied *Lactobacillus mudanjiangensis* and its industrial potential

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In 2016, the first *Lactobacillus mudanjiangensis* strain was isolated from a Chinese pickle fermentation. This species belongs to the prominent *Lactobacillus plantarum* group, that is known to be present in fermented foods, silage, and mucosa of both humans and animals. Recently, an additional three strains of this food-grade and safe bacterium were found in Flemish carrot fermentations during the citizen science project Ferme Pekes.

Computational analysis of the four currently discovered *L. mudanjiangensis* genomes predicted the presence of a number of unique features within the *Lactobacillus* Genus Complex, which harbor a great potential economic impact. These features need further validation and additional research (Wuyts et al., 2019). One of these features was a putative cellulose-degrading enzyme termed cellulase. Cellulases are used in various industries including the production of bioethanol, coffee production, and recycling of cellulose-containing material. The presence of cellulases in lactic acid bacteria that are safe to use in food and pharma industry, could positively impact safety and wellbeing regulations. This safety aspect is important since different industries have recurrent problems of occupational exposure of the employees to gram-negative bacteria (more specifically, their LPS-derived endotoxins) and fungi, and their associated health risks. In this study, the novel cellulase was characterized and heterologously expressed to determine its functionality and specificity.

Wuyts, S., Allonsius, C. N., Wittouck, S., Thys, S., Lievens, B., Weckx, S., ... Sarah, L. (2019).

Comparative genome analysis of *Lactobacillus mudanjiangensis*, an understudied member of the *Lactobacillus plantarum* group. *Microbial Genomics*.

## Molecular engineering applied to the directed synthesis of fengycin by *Bacillus subtilis*

Quentin GRAS<sup>1,2</sup>, Frédérique GANCEL<sup>1</sup>, François COUTTE<sup>1</sup>, Valérie LECLERE<sup>1</sup>, Philippe JACQUES<sup>2</sup>

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Lipopeptides produced by *Bacillus subtilis*, like surfactin or fengycin can be an alternative to chemical pesticides in agriculture. Fengycin is a promising antifungal lipopeptide from *Bacillus* spp. synthesized by non-ribosomal peptide synthetases (NRPS) however, its productions by wild strains are too low to be an economically viable solution to its massive use of chemical pesticides. Genetic enhancement of lipopeptides production has gained attention in the last years and for that, different strategies can be followed.

In *B. subtilis* 168 the promoter of the fengycin operon,  $P_{fen}$  showed low efficiency. In this work, in order to improve fengycin operon expression, this promoter has been substituted by a panel of 6 strong promoters: the surfactin operon promoter  $P_{srf}$  and two partial but functional surfactin operon promoters  $P_{srf\_84}$  and  $P_{srf\_89}$  (1) ; the two strong constitutive promoters from *B. subtilis*  $P_{veg}$  and  $P_{43}$  (2); and the strong constitutive one from *Staphylococcus aureus*  $P_{repU}$  (3). The production of fengycin of the mutant strains so obtained has been studied and the best results have been observed with the strain whose the *fen* operon is under control of  $P_{srf\_89}$  with a production of fengycin 6 times more important compared to the mother strain.

Many regulators control the fengycin production. Among all of them, four were selected because they negatively impact *fen* operon expression. First, *AbrB* is a transcriptional regulator of transition state genes which represses the expression of the *fen* operon and *phoP* (a gene activating the expression of the *fen* operon) (4,5). *CodY* participates in the regulation of a large regulon (more than 100 genes and operons) in response to branched-chain amino acid limitation. The decrease in its expression increases that of *degQ* (6–8) which positively impact *fen* expression (9). Then, *ScoC* is a transcriptional repressor of genes expressed in the transition phase which can repress the *PhoPR* system that positively regulate *fen* expression (10). At last, *SinR*, a transcriptional regulator of post-exponential-phase response genes, interact directly with the regulatory region of the *fen* operon and indirectly by repressing the expression of *degU* (11,12). The genes coding for those regulators were knocked-out and the lipopeptides productions of the respective mutants have been studied. Fengycin, productions have been increased by 1.6 times for  $\Delta codY$  mutants and 2.4 times for  $\Delta sinR$  or  $\Delta scoC$ . The largest production increase has been obtained with  $\Delta AbrB$  mutant that produced 3.6 times more than the mother strain.

Those results showed that fengycin synthetase native promoter is partially responsible for the low productions of this lipopeptide by *B. subtilis*. Nevertheless, substitute it by a stronger one or acting on the global regulation can improve those productions.

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## Session 2 (ZT1)

# Climate change

14h00 -15h00

**Chairman: Christian Hermans – ULB**

The Use of Renewable Hydrogen for Maritime Applications  
Laurens Van Hoecke (UAntwerp)

Effects of high temperature on plant reproduction and health-promoting qualities of two buckwheat species *Fagopyrum esculentum* and *Fagopyrum tataricum*  
Lauranne Aubert (UCLouvain)

Integrating dynamic energy budgets in a spatially explicit individual-based framework  
Wissam Barhdadi (UGent)

Potential impacts of climate change in larval development and oviposition choice of an aphidophagous hoverfly species *Episyrphus balteatus* (Diptera : Syrphidae)  
Grégoire Noël (ULiège)

Flash presentations

## Emergy Analysis of Typical Straw Utilization Mode: Case Study in Hebei Province

Qiang Wang <sup>a,b</sup>    Thomas Dogot <sup>b</sup>    Guosheng Wu <sup>a</sup>    Changbin Yin <sup>a,c,\*</sup>

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### Abstract

China's coal-based energy structure is the main reason for the current high-level air pollution and carbon emissions. Now in the North China Plain, the government is vigorously promoting “coal to gas” and “coal to electricity” in the county and the vast rural areas. The development and utilization of biomass resources in agricultural areas is also an effective means of replacing coal. This paper selects two most commonly straw energy utilization modes, large and medium-sized centralized biogas production mode and straw briquetting fuel mode, combines with the actual investigated on-going project cases, uses emergy analysis method to analyze. It is found that under the current capacity load, the eco-economic indicators, sustainable development indicators, environmental load indicators have their own advantages and disadvantages. Compared with coal power, both models have high renewability and low environmental load. The two models are in full compliance with the environmental protection requirements of the current rural ecological civilization construction in terms of environmental indicators. But it does not have long-term sustainability because economic indicators and sustainability indicators are not good. Especially in the case of severe low load capacity, the economic and sustainability of the two models are relatively poor, but with the release of production capacity, assuming 100% capacity load, the economics and sustainability are greatly improved. A certain gap needs to increase government intervention and support. In mode selection, it can take strengths and avoid weaknesses in accordance with the characteristics of the model itself. The process of the analysis reveals the improvement measures of the two modes, there is still great optimization space in project management, technical process, market development and so on. Thereby, we can explore the optimization path of rural energy structure in Hebei Province even the whole North China Plain to realize the energy utilization of rural biomass resources. Through the gasification and solidification of biomass energy, and the integration and utilization of electricity, hybrid renewable energy system, the optimal path of domestic energy use in rural areas is sought under the premise of economic

and environmental sustainability.

**Keywords**

Emergy Analysis; Centralized Biogas Production; Straw Briquetting Fuel

# Residents' Willingness for Centralized Biogas Production in Hebei and Shandong Provinces

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## Abstract

Nowadays, in the North China Plain (NCP), most counties and suburban rural regions are promoting the "coal to gas" program. Natural gas and large and medium-sized centralized biogas production (CBP) are all supported by the government. This paper is aimed at the CBP projects, focuses on rural residents' "Willingness to Participate (WTPA)", "Willingness to buy (WTB)" and the influencing factors for WTPA/WTB. Based on the survey data collected in Hebei and Shandong provinces in 2018, this study estimated farmers' WTPA/WTB for CBP projects and determined the factors affecting farmers' decision-making. We find that 85% of the respondents support the CBP project, the WTB is 63.15 CNY/year per capita. The gap between WTB and gas bills, WTB and production costs are still very obvious. The high proportion of WTPA is mainly due to a series of administrative measures currently taken for environmental governance. The family characteristics such as the number of family members and the number of children in the family are highly correlated with WTB. The WTPA/WTB is not only a manifestation of personal and family characteristics, understanding of environmental knowledge, attitudes and household energy practices are more important. The non-economic factors such as residents' knowledge, attitudes, and practices for environmental protection have significant impacts on WTB. These non-economic factors seriously affect WTPA/WTB. These non-economic factors can improve the residents' WTB by providing environmental education to residents through television and other modes of guidance. This will reduce the subsidy rate and enhance the sustainability of the CBP project. Thence, it is necessary to appropriately increase the government input to such projects, strengthen government guidance and publicity, improve the environmental knowledge and attitude of rural residents and guide residents' practice. Based on a fully understanding of residents' consumption decision mechanism, establishing a subsidy mechanism due to the demand response, adopting a reasonable subsidy rate,

seeking the greatest commonality of cost, subsidy, and demand.

**Keywords**

Centralized Biogas Production (CBP); Contingent Valuation Method (CVM); Heckman Selection Model; Willingness to Participate (WTPA); Willingness to Buy (WTB)

**Potential impacts of climate change in larval development  
and oviposition choice of an aphidophagous hoverfly species  
*Episyrphus balteatus* (Diptera : Syrphidae)**

Grégoire Noël, Jessica Caetano, Solène Blanchard, Frédéric Francis

Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Belgium

Numerous studies have shown that climate change could, directly or indirectly, contribute to disturb insect's biology and interactions with plants. An increase of mean temperatures and atmospheric carbon dioxide concentration is known to have various effects on insects-pests biology, behavior and communication. However, the impact of climate change on their natural enemies is still poorly understood. Following IPCC temperatures and CO<sub>2</sub> predictions for the next decades, we first investigated (i) the impact of an elevation of temperature, here 20°C, 23°C and 26°C on the weight, size, and consumption in aphid (*Acyrtosiphon pisum* Harris), of the larvae development of the hoverfly *Episyrphus balteatus* (De Geer). We also tested (ii) the hypothesis that a combination of CO<sub>2</sub> concentration (450 and 800ppm) and temperature (20 and 23°C) may affect the oviposition choice of *E. balteatus* gravid females on bean plant (*Vicia faba* L.) infested with aphids. Dual-choice bioassays were used for each temperature and CO<sub>2</sub> combination. We found that temperature has no effect on the larval development of *E. balteatus*, but has an effect on the weight and size of the final larval stage. Furthermore, contrary to CO<sub>2</sub> concentration, an elevation of temperature also has an effect on the site choice and number of eggs layed on the plants. Our results suggest that an elevation of temperature has an impact on hoverfly biology and oviposition. Temperature may impact plant volatile profile, as well as aphid honeydew, indirectly impacting hoverfly attraction. These outcomes offer new overview about multitrophic interactions under worldwide climate change.

## Using remote sensing satellite observations to assess the phytoplankton response to extreme climatic phenomena in the waters surrounding Cuba

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<sup>1</sup>Universidad Central "Marta Abreu" de Las Villas, Cuba

<sup>2</sup>Ghent University, Belgium

An understanding of the marine phytoplankton response to hurricanes and the El Niño Southern Oscillation (ENSO) is of importance for studies on climate change, ecological variability, and environmental protection. Despite the peculiar oceanographic characteristics of the waters surrounding Cuba and the fact that they are highly affected by hurricanes, research on phytoplankton is scarce. In this study we assess for the first time the response of the chlorophyll-a (chl-a) concentration (as a proxy for the abundance of phytoplankton) to hurricanes and strong ENSO events in the waters of the Cuban Exclusive Economic Zone (EEZ) in the period 1998–2017 using satellite observations as primary source of information. Overall, hurricanes induced a chl-a increase during the first two post-storm weeks, with daily spatially averaged anomalies ranging between 0.016 and 0.034 mg m<sup>-3</sup> along the hurricane trajectory. This post-storm bloom was driven by the transport of chl-a from the deep chlorophyll maximum (via vertical mixing and upwelling) and the horizontal advection of chl-a rich waters from the Cuban coast and remote regions of the Gulf of Mexico. On the other hand, strong ENSO events in 1998 and 2016 led to the highest monthly chl-a anomalies (i.e., 0.044 and 0.046 mg m<sup>-3</sup>, respectively) over the entire Cuban EEZ. This increased biological response was linked to active wind regimes (modulated by successive cold fronts) and deep mixed layers.

## **Assessment of Walloon dairy farms eco-efficiency using Data Envelopment Analysis and easily-accessible environmental and economic indicators: a preliminary study**

**Delhez P.<sup>1,2</sup>, Reding E.<sup>3</sup>, Gengler N.<sup>2</sup>, Soyeurt H.<sup>2</sup>**

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Achieving economically viable and environmentally friendly food production is a key challenge today. In this context, the aim of this study was to (i) analyse the economic and environmental efficiency (i.e., eco-efficiency) of a sample of specialised dairy farms in the Walloon region of Belgium; and (ii) to identify key management factors that differ between efficient and inefficient farms. Eco-efficiency was estimated with the productive efficiency benchmarking method Data Envelopment Analysis (**DEA**). DEA is a well-known technique for measuring the relative efficiency of comparable decision-making units using several inputs to produce one or more outputs. In our study, input and output variables were selected based on their economic and environmental relevance, as well as on their availability in the accounting database of the Walloon Breeding Association (awé). The chosen DEA inputs and output included economic-oriented variables such as fat and protein corrected milk yield and simple environmental indicators like land use, livestock units, fertiliser and pesticide application, purchased feed and on-farm energy use. Preliminary results on 174 dairy farms in 2017 suggested contrasting levels of eco-efficiency in our sample. Hypotheses concerning the determinants of eco-efficiency will be tested. The findings of this study will help inform policy-making towards dairy farm management that can increase dairy production at the least environmental costs.

## High CO<sub>2</sub> mitigates drought stress in maize leaf by increasing soluble sugar in leaf meristem and maintaining high sink strength

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### Abstract

Given the economic importance of C<sub>4</sub> plants, it is important to understand the individual and combined effect of future climate conditions. To provide a substantial contribution to this understanding, we used the developmental gradient of the maize leaf as a model system, comparing proliferating, expanding, and mature leaf tissues of plants developed under drought stress and/or eCO<sub>2</sub>. Kinematic analysis indicated that eCO<sub>2</sub>-treated reduced drought stress impact on cell division and cell elongation. At the transcriptomic level, eCO<sub>2</sub> stimulates drought-induced increase in photosynthetic machinery in mature cells and sugar metabolism and the antioxidant and redox systems in the growth zone. eCO<sub>2</sub> mitigates drought stress by increasing soluble sugars and antioxidants in leaf meristem. We demonstrated the functional significance of the identified transcriptional and metabolic reprogramming by showing that reducing the eCO<sub>2</sub> impact on soluble sugars level, by down-regulation of the expression of sucrose synthase gene (*sh1*), eliminated eCO<sub>2</sub>-stimulating cell division rate in meristematic zone. On the other hand, supplying growing leaves, at the first day after emergence, with sucrose (0.3M) through their cut ends increased meristem length and leaf growth rate. Overall, this study contributes to our understanding of the effects of elevated CO<sub>2</sub> on stress responses in C<sub>4</sub> plants, in particular on the underlying molecular and cellular mechanisms.

# Integrating dynamic energy budgets in a spatially explicit individual-based framework

Wissam Barhdadi<sup>1</sup>, Aisling J. Daly<sup>1</sup>, Jan M. Baetens<sup>1</sup>, Bernard de Baets<sup>1</sup>

<sup>1</sup>KERMIT, Department of Data Analysis and Mathematical Modelling, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

Ecological systems are characterized by their complex dynamics. Understanding these dynamics is a challenge, yet important for various applications such as predicting the impact of changing environments. Individual-based models (IBM) have proven successful at contributing to this endeavour. In ecology, IBMs are based on rules involving an individual organism's life-history traits and behaviour. Even though in recent years the IBM framework has become an established tool for ecological modellers, the implementation of the rules for individual organisms remains highly variable and situation-specific.

Dynamic energy budget (DEB) theory provides a formal and quantitative description of the intake and distribution of energy within an individual organism [1]. It thus describes multiple aspects of the physiological life-history in an integrative manner while abiding by the principles of energy and mass conservation. DEB and IBMs are highly compatible since both are defined at the level of the individual, yet little attention has been paid to developing a coupling between them. Such a coupling would allow simulating ecological systems grounded in energetic principles while accounting for energy-based life-history trade-offs and dynamic environments.

We propose an integration of the standard DEB model with an IBM framework. In a first approach, both models are implemented as they are established within their separate fields. To assess the combined model's behavior, simulations of a biological population under different temperature and within-population diversity regimes are performed. From this first implementation, some conceptual gaps became clear. However, further improvements to this integration will allow for a promising and novel approach for modelling complex ecological systems.

## References

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Title :
High-moisture pelleting as an energy-efficient alternative to conventional pelleting
Authors :
Thibaut Masy <sup>1</sup> , Olivier Hecq <sup>1</sup> , Bruno Godin <sup>1</sup> , Jérôme Delcarte <sup>1</sup>
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Abstract :
<p>In the frame of reducing greenhouse gas emissions and achieving carbon neutrality, the development of bioenergy, <i>i.e.</i> the use of biomass to produce fuel or energy, is ongoing. Given the heterogeneity and the low density of biomass, pelletizing appears as an efficient process to compact biomass, in order to facilitate transport, storage, handling, supply and finally combustion. However, the grey energy related to this process is rarely quantified and can be reduced.</p> <p>The objective of our study is therefore to optimize the energy consumption and pellet quality, by assessing the critical parameters involved in pelleting (humidity, compression, feeding rate). The biomass studied here is a spruce sawdust coming from a local sawmill. Our strategy aims at pelleting untreated sawdust (high-moisture pelleting), to limit energy costs from drying (performed in conventional pelleting), as shown by Lamers et al. (2015).</p> <p>Our experiments presented here compared 3 moisture contents and 3 feeding rates of sawdust with a lab pelleting press (3 kW), using two different ring dies (single and double rows). Based on these variables, the electrical consumption and temperature of the press, as well as the moisture content, production rate, fraction of fines and durability of the pellets were measured in order to determine the quality of the process and the resulting pellets.</p> <p>First results showed that a moisture content of sawdust between 20 and 26% (humid basis) is an acceptable range for an energy-efficient pelleting process (compared to 8 to 14% recommended for conventional pelleting). However, the pellet quality need to be slightly improved to meet ISO standards, especially when the feed rate and humidity are the highest.</p> <p>Reference: Lamers et al. (2015). <i>Techno-economic analysis of decentralized biomass processing depots</i>. Bioresource Technology, 194, 205–213.</p> <p>Acknowledgment: This work was performed as part of the ENERBIO project, supported by a FEDER fund.</p>

## Beyond turbulence, nonlinearity and chaos: on the nature of Earth's complexity

Sergio Rubin, UCLouvain, Belgium

Current appraisals of what Earth's complexity is tends to focus on turbulence, nonlinearity and chaos, all them amenable to algorithmic procedures. The present article attempts to place the nature of Earth's complexity on a modelling framework based on non-algorithmic biological characterizations such as the metabolic, repair (M,R)-system and autopoiesis. We compare how Earth's complexity looks like in reference to the Earth computable simulations such as general circulation models and chaotic systems, and explore the computational challenges that this implies. That is, rather than enumerating the changes in the set of physical quantities that are encoded in the state vector, Earth's complexity appears to be based on causal relationships of Earth's climate components within a defined organisation of self-fabrication by closure to efficient causation. This poses challenges for climate simulators, on which relations that may take place between distant Earth's components on the planet is supposed to be reducible to parameters in grid cells. Yet, it opens the possibility of laying down a research programme to understand the Earth dynamics in biological terms.

Submitted for poster

Comparison of salinity response of the cultivated tomato (*Solanum lycopersicum*) and its wild relative *Solanum chilense* during reproductive stage

Bigot, Servane<sup>1</sup>; Martinez, Juan-Pablo<sup>2</sup>; Lutts, Stanley<sup>1</sup>; Quinet, Muriel<sup>1</sup>

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Abiotic stress as salinity affect glycophyte plants such as tomato (*Solanum lycopersicum*) at the whole-plant level. Although soil salinity is a growing problem in the world and tomato is the second most highly produced vegetable worldwide, little is known about traits that influence salt resistance at reproductive stage in this species. This stage is often more sensitive to abiotic stress than vegetative stage and represents a major issue for tomato industry. Resistance to salinity is a multi-gene mechanism and de novo domestication of wild relative species could be an efficient strategy to improve salt-resistance in tomato. The wild tomato *Solanum chilense* is able to cope with harsh environment and represents an abundance of genetic diversity for tomato improvement. However, physiological basis of salinity tolerance in *S. chilense* remains largely unknown. The aim of our research is to compare the changes mediated by salinity in *S. chilense* and *S. lycopersicum* during vegetative and reproductive stages. Plants of *S. chilense* and *S. lycopersicum* were grown under greenhouse conditions under three concentrations of salt: 0, 60 and 120 mM NaCl. Regarding vegetative parameters, salinity decreased aerial dry weight of both species. Regarding reproductive parameters, salt decreased the number of inflorescences and affected flower morphology in both species. NaCl reduced fruit dry weight only in *S. lycopersicum*. Regarding sodium concentration in the different organs, both species accumulated less Na in inflorescences than in vegetative parts. However, the inflorescences of *S. lycopersicum* accumulated more Na than the ones of *S. chilense*. Our results suggest thus that both species may differ in their sensitivity to NaCl at reproductive stage.

References :

Böndel KB, Nosenko T, Stephan W (2018). R. Soc. Open Sci. 5: 171-198 ; Rodriguez-Ortega WM, Martinez V, Nieves M, Simon I, Lidon V et al (2019) Sci Rep 9:6733; Zsögön A, Cermak T, Voytas D, Peres LE (2017) Plant Sci. 256:120-130

## **Submission as a poster**

### Comparison of drought and heat resistance strategies among 6 populations of *Solanum chilense*.

Rémi Blanchard-Gros<sup>a</sup>, Juan-Pablo Martinez<sup>b</sup>, Muriel Quinet<sup>a</sup>

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<sup>b</sup> Instituto de Investigaciones Agropecuarias (INIA – La Cruz), La Cruz, Chile

Unfavourable environmental conditions such as drought and high temperature significantly limit plant growth and productivity in Tomato (*Solanum lycopersicum*). However important sources of variation for this stress tolerance exist in wild relatives like *S.chilense* and the elucidation of tolerance strategies in this species living in dry zones is a powerful approach to understand local adaptations for potential crop improvement and genetic diversity. The aim of our research is to study the changes mediated by drought and heat acclimation in *S.chilense* in order to highlight morpho-physiological basis of stress tolerance in this species during vegetative and reproductive stages. Six populations of *S.chilense* belonging to different phylogenetic groups and growing in contrasted habitats and two cultivar of *S.lycopersicum* were compared. Plants were grown under controlled conditions and 4 conditions were applied : 2 temperatures conditions (28 and 21°C) and 2 water status (100 and 50% field capacity). Morpho-physiological results allowed to determine 3 groups of accessions according to their tolerance or sensitivity to the stress conditions. The number of leaves and the plant size of sensitive accessions were reduced by 25 - 40% under water stress compared to controls. The resistant accessions did not show any difference between treatments. Flowering time was delayed in plants grown at 28°C compared to 21°C. The physiological parameters (chlorophyll fluorescence, gas exchanges, etc.) differed more among accession than among treatments. The expression of 20 stress-related genes was analyzed in the different accessions under high temperature and water stress and the results were correlated with the morpho-physiological parameters.

## **Vulnerability assessments in dairy cattle farms based on individual sensitivity to heat stress**

Please supply your title here (max. 250 characters)

Please include here the list of authors and their affiliations

Please supply your abstract here (max. 1500 characters)

Please include here the references (please keep to the minimum necessary)

Hajer Amamou<sup>1</sup>, Mokhtar Mahouachi<sup>2</sup>, Yves Beckers<sup>1</sup> and Hedi Hammami<sup>1</sup>

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<sup>2</sup>Higher School of Agriculture of Kef, University of Jendouba. 7119 Le Kef, Tunisia

Climate change is expected to increase temperatures and the frequency of extreme weather events, which renewed interest in heat stress (HS) effects on dairy cattle farms. This study aimed at evaluating the impacts of HS on milk production in Holstein cows to investigate the aspects of vulnerability to climate change in Tunisia. Milk test-day records from official milk recording for the period between 2005 and 2012 were merged with temperature and humidity data provided by public weather stations. Values for the daily average and maximum temperature-humidity index (THI) were calculated. Different models relying in these two indices to measure heat load are used to explore average patterns of response of milk production performance to HS. A broken line model was used to estimate HS thresholds and rate of decline of milk production associated with THI across parities. Tolerant and sensitive cows to HS were identified based on their slope for milk yield response provided by reaction norm test-day models. Farm vulnerability to HS was defined based on five indicators of climate sensitivity to review how dairy cattle farms might be vulnerable to climate change. Values for the daily average and maximum THI ranges were 45 to 80 and 50 to 85, respectively. The tipping points, at which milk yield started to decline over parities with 3-d average THI ranged between 65 and 67. The high sensitivity of Tunisian Holstein cows to HS were proved especially in dairy farms characterized by large herd size and high level of production. Hence, providing knowledge of the different ways in which dairy farms can be vulnerable to HS may provide the basis for developing strategies to reduce effects of HS and plan for climate change adaptation.

## The Use of Renewable Hydrogen for Maritime Applications

Laurens Van Hoecke – Sustainable Energy, Air and Water Technology,  
University of Antwerp

Sammy Verbruggen – Sustainable Energy, Air and Water Technology,  
University of Antwerp

Silvia Lenaerts – Sustainable Energy, Air and Water Technology, University of  
Antwerp

In this research different methods for H<sub>2</sub> storage are compared to each other and their usefulness for the maritime industry is evaluated. The selection of a fitting storage method for H<sub>2</sub> is crucial in the development of a more sustainable maritime industry. Currently, the maritime industry is responsible for 2 – 3 % of all global CO<sub>2</sub> emissions, totaling close to 10<sup>9</sup> ton of CO<sub>2</sub> annually<sup>1</sup>. This CO<sub>2</sub> is caused by the emissions of fossil fuels, mostly Heavy Fuel Oil, a heavy fraction of crude oil distillate.

In business-as-usual scenario's the CO<sub>2</sub> emission from shipping is expected to rise by 50 – 250 % in 2050<sup>1</sup>. Therefore, it is crucial that new technologies are introduced for a more sustainable shipping industry<sup>2</sup>. One such new technology could be the use of H<sub>2</sub> which can be a zero-emissions fuel if it is generated via electrolysis with renewable energy. Being a very light gas, H<sub>2</sub> has a very low power density (at ambient conditions, roughly 3300 times lower than diesel), and thus there lies a significant challenge in concentrating hydrogen to reasonable levels. In this research an assessment is made for H<sub>2</sub> stored in gas tanks, in liquid form, in methanol, in ammonia and in liquid organic hydrogen carriers and they are assessed based on their safety, availability and ease of use in the maritime industry.

1. Smith, T. W. P. *et al.* *Third IMO Greenhouse Gas Study 2014*. (2015).
2. Gilbert, P. *et al.* Assessment of full life-cycle air emissions of alternative shipping fuels. *J. Clean. Prod.* **172**, 855–866 (2018).

## Effects of high temperature on plant reproduction and health-promoting qualities of two buckwheat species *Fagopyrum esculentum* and *Fagopyrum tataricum*.

Lauranne Aubert<sup>a</sup>, Daniela Konrádová<sup>b</sup>, Salima Kebbas<sup>c,d</sup>, Selma Barris<sup>d,e</sup>, Muriel Quinet<sup>a</sup>

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In the context of ongoing climate change, expected temperature rise may significantly limit plant growth and productivity of crop species. In this study, we investigated the effects of a sub-optimal temperature on buckwheat, a pseudocereal known for its nutraceutical advantages. Two buckwheat species differing by their reproduction method, namely *Fagopyrum esculentum* and *Fagopyrum tataricum* were grown at 21°C and 27°C in growth chambers. High temperature increased leaf production mainly in *F. tataricum* but decreased leaf area in both species. Regarding reproductive stage, high temperature delayed flowering time but boosted inflorescence and flower production. Nevertheless, flower and seed abortion were observed in both species at 27°C. Regarding flower fertility, heat affected more the female stage than the male stage and reduced the stigma receptivity. Pollen production increased with temperature in *F. esculentum* while it decreased in *F. tataricum*. Such discrepancy could be related to the self-incompatibility of *F. esculentum*. Both species increased their antioxidant production under high temperature to limit oxidative stress and antioxidant capacity was higher in the inflorescences than in the leaves. Total flavonoid content was particularly increased in the leaves of *F. esculentum* and in the inflorescences of *F. tataricum*. Altogether, our results showed that even if high temperature may negatively affect reproduction in buckwheat, it improves its antioxidant content.

## **A comparison of the behaviour of wheat crops submitted to two meteorological conditions representative of the actual climate and of a possible future climate by using an Ecotron**

A. Saré, A. Anckaert, A. Degré, B. Dumont, B. Mercatoris, B. Bodson, B. Heinesh, B. Longdoz, C. Thonar, C. Gilles, F. Zahra, F. Williscotte, F. Verheggen, J. Fouché, G. Duhaubois, H. Soyeur, H. Vanderschuren, J.-T. Cornelis, J. Pierreux, K. Wijnroox, L. Willems, M. Antoine, M. Ongena, P. Malumba, P. Dujardin, P. Jacques, P. Delaplace, R. Hamdi\*, S. Garré, S. Massart, V. Leemans, Y. Brostaux

Tou sauf \* : Terra, Gembloux Agro-Bio Tech, Liège University

\* : Royal meteorological institute of Belgium

In this experiment a winter wheat crop is submitted to two meteorological conditions. The actual conditions (harvest year 2015) were applied in three rooms while the three others were submitted 2094 conditions, representative the period 2070-2100 according to RCP 8.5 (Alaro0). The crop has been placed in a circular lysimeter of 2 m<sup>2</sup> horizontal area and 1.5 m depth where bottom conditions are also controlled.

Comparison with field measurements in 2015 showed that an important “Ecotron effect” was observed, with an advance in the juvenile and vegetative phenological stages but not at maturity. The leave development was also higher in the Ecotron. Consequently, the yield was 50% higher in the Ecotron. The long wave radiation balance and the wind might be questioned.

Unless otherwise stated, the quantities describing 2094 are compared to those of 2015. The phenological stages were in advance, particularly during tillering (December to February) which was 29 days shorter. The maximal daily air temperature was often above 10°C. The number of tiller was smaller, 3.9 against 5 but the green area index was more than twice as high. The evapotranspiration was thus higher and the soil humidity profile dryer in the first 20 cm (-15 l.m<sup>-2</sup>). During the elongation and booting, nothing particular was observed. The conditions changed during the anthesis in 2094, becoming warmer (day t° ≥ 25 °C) with scarce precipitations. During the development of the fruit and maturation the crop was in water stress. The chlorophyll fluorescence and evapotranspiration which have consistently been higher in 2094 dropped during this period. This clearly affected the yield which was 33% lower. More results are available and may be presented.

## Session 3 (BV)

# Urban ecosystem, forest ecology and management

15h30 -16h30

**Chairman: Arnaud Monty – ULiège**

Flexible habitat use of an open-habitat bird species in a farmland-woodland landscape of southern Belgium

Robin Gailly (ULiège)

The impact of information-transfer related to soil biodiversity on Flemish citizens' preferences for forest management

Iris Vanermen (KULeuven)

The invasive potential of alien conifers assessed in old Belgian arboreta

Aurore Fanal (ULiège)

Modeling the reduction in sapling growth, density and diversity along a gradient of wild ungulate density

Romain Candaele (ULiège)

Flash presentations

## **Integrating ecosystem services and resilience in sustainable forest management**

Laura Maebe<sup>1</sup>, Hugues Claessens<sup>1</sup>, Marc Dufrêne<sup>1</sup>, Kevin Maréchal<sup>1</sup>, Christian Messier<sup>2</sup>

1 - Gembloux Agro-Bio Tech, University of Liège, Belgium

2 - Institute of Temperate Forest Science (ISFORT), University of Québec in Outaouais, Canada

In the Anthropocene era, humanity has considerably altered the functioning of Earth, resulting in global and inter-related social, economic and environmental crises. In response, resilience, ecosystem services (ES) and sustainability have gained tremendous popularity in the scientific, policy and management arenas. However, less attention has been paid to the relationships between ES and resilience and how these concepts interact with sustainability. We, therefore, analyzed the concepts of ES and resilience, their relationships, strengths and weaknesses to determine how resilience and ES could be together operationalized for sustainable forest management.

This analysis, based on a literature review and on interviews with experts, shows that resilience and ES are closely intertwined. They meet in the social-ecological system perspective where resilience determines the capacity of the system to face disturbances and thus to provide ES and is influenced, in turn, by human actions taken to response to changes in ES. In a narrower sense, resilience is defined as the ability to maintain ES. Finally, in some ES classifications, resilience is treated as an ES among others.

The resilience approach contributes to improve the ES approach and vice versa: resilience introduces the temporal dimension in ES while ES help integrating the multiple dimensions, scales, methods and points of views as well as their interactions in resilience. Resilience may be mandatory to ES and vice versa as a loss of resilience/ES could jeopardize ES/resilience. In conclusion, pairing ES and resilience is essential to promote policies toward sustainable forest management. However, caution should be exercised to avoid traps of one concept overriding the other.

## Inventory of ant species in the Marseilles suburbs

Gabriel OLIVE, Gilles OLIVE and Wouter DEKONNINCK

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Marseilles is a big city with several ecological areas, like pure urban, suburban, rural areas (mainly Garigue which is a type of low, deciduous Mediterranean forests). The knowledge on ant distribution in this particular region is limited (1;2). Moreover one of the so far known lists, only deals with ant communities of some islands near Marseille. To increase the knowledge on ant distribution, several sites in east Marseille were selected and different habitats, mainly suburban and Garigue were sampled for ants. Two methods to collect ants were used: hand sampling (HS) and pitfall trapping (PF) (without and with attractive i.e. sweet white wine).

Here the first results of this study and the species found are presented. *Aphaenogaster splendida* and *Messor capitatus* were observed for the first time in Marseilles and populations of these two species will be monitored during the next years.

- 1 AntArea Etude, identification, répartition, localisation des fourmis françaises métropolitaines. **2019** (<http://antarea.fr/>; Accessed 5<sup>th</sup> november 2019).
- 2 Berville, L.; Renucci, M.; Vidal, P.; Provost, E. Peuplement myrmécologique et évaluation de l'invasion de *Linepithema humile* sur les îles de Marseille (Bouches-du-Rhône, France). *Revue d'Ecologie (Terre et Vie)* **2016**, 71, 278-287.

Comparison of methods to model species habitat networks for decision-making in nature conservation: the case of the wildcat in Southern Belgium.

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Facing the loss of biodiversity caused by landscape fragmentation, implementation of ecological networks connecting habitats through landscapes is an important biodiversity conservation issue. Yet, it is necessary to develop easily reproducible methods to identify and prioritize actions to maintain or restore ecological corridors. We tested three different methods of identifying species corridors. We aimed to identify the parameters that affect most of the results by comparing knowledge-driven (expert based), data-driven (based on species distribution model) and mixed approaches. To put this into practice, the case study of the wildcat (*Felis silvestris* Schreber, 1777) was chosen. The results showed that the different methods provided different priority action maps. The data-driven approach was more successful in identifying the suitable habitat with regard to wildcat ecology while the knowledge-driven approach was better to account for obstacles to wildcat movements in the landscape matrix. However, these two methods identified similar pattern of habitat patches and corridors that are important for the global landscape connectivity. The mixed approach largely differed while it requires more inputs to be performed, and was then dismissed. We conclude that, for this study, the data-driven approach gave the most relevant results with the most reproducible method. We still suggest improving this approach by enhancing the resistance of blocking elements based on expertise. We highlight that the parameter affecting the results the most was the dispersal distance used to model corridors. We therefore advise performing sensitivity analyses on this parameter to estimate associated uncertainty.

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**Increasing the biodiversity of extensive green roofs using native plants is not so easy. Exploring the plant community responses to substrate depth and insolation.**

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Extensive green roofs (ExGR) are part of green infrastructures and present an opportunity to support native biodiversity. However, most of existing ExGR in Europe support exotic stonecrop with poor diversity. In term of structure, the abiotic conditions of the ExGR substrate present analogies with dry grassland: poor, shallow and highly drained soil. This study aims to understand vegetation dynamics of sown seed mixtures which include two native plant communities (xeric and mesoxeric) on two substrate depths and three insulations to test the opportunity to create analogous habitats. A total of 29 native species were used. We used the much used ExGR substrate based on recycled bricks. The plant community's structure and main CSR strategies were measured over 2 years.

The results showed that the soil depth and the insolation promoted the creation of distinct plant communities over time. The specific abundance and richness increased over time and were higher in plots with less insolation and higher substrate depth. Plant community cover was higher in the most exposed plots with higher substrate depth. Insolation and substrate depth had also modified CSR strategy of the plant community. A deeper substrate and a lower insolation promote a community characterized by a S-R strategy. When the insolation increased and the substrate depth decreased, the plant community tended to be more ruderal (R).

Based on this study, we show that the implementation of analogous habitat of dry grassland still should be improved by taking into account the heterogeneity of micro-environmental conditions on a roof. Further studies should better consider the structure of ExGR in order to create a less stressful habitat and better adapt the plant selection.

Key words: Dry grassland, nature-based solutions, biodiversity, plant community dynamic

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# The invasive potential of alien conifers assessed in old Belgian arboreta.

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An increasing number of tree species are being listed as invasive in Europe. Forestry is the second largest pathway of alien trees introduction and the phenomenon is likely to increase. In the early 1900's, 23 arboreta were established in Southern Belgium to assess the wood production potential of prospective alien trees, including many conifers. Today, they offer the unique opportunity to assess the potential invasiveness of these trees.

We quantified the invasive potential of coniferous species in eight arboreta. Using a systematic sampling, the regeneration of trees as well as environmental variables were characterized. For each species, regeneration density, dispersal distance and age structure were then analyzed. A principal component analysis was used to identify the environmental conditions in which each species regenerated most.

Seven species, mainly originating from the North-American West Coast, exhibited rapid regeneration and dispersal, especially *Tsuga heterophylla* and *Abies grandis*. *Chamaecyparis lawsoniana*, *Thuja plicata* and *Pseudotsuga menziesii* were also found in high densities in three arboreta. Environmental analysis showed that *T. heterophylla* exhibited more regeneration on moist, acidic soils, while *A. grandis* was found on dryer and more basic soils. Our study demonstrates that introduced conifers can show an invasive behavior and may threaten native ecosystems. We recommend exercising caution when planting alien species in future forestry trials.

## Do alien plants get adapted to urban environments? A case study with pineapple weed.

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Urban environments host many alien plant species and represent particular growing conditions. They display warmer and drier climate than the rural surroundings, because of a high proportion of built up surfaces and modified soils. They also tend to have a lower connectivity than rural areas, which can lead to the isolation of populations. This makes urban environments useful systems to study alien plant evolution, especially because these areas somehow mimic future conditions under the ongoing global changes: warmer climate and increased anthropogenic activities.

We used *Matricaria discoidea* DC. as model species and selected 16 populations along urban-rural gradients in Belgium. With the help of Copernicus imperviousness layer, we characterised their growing environment at the local (20m) and at the landscape scale (3km), either as urban (>10% imperviousness surfaces) or as rural (<10% imperviousness surfaces).

In two split-plot design experiments, one outdoor and one in controlled growth chambers, we investigated the effects of microclimate (urban/rural), soil (urban/rural), and population origin (urban/rural) on the development and fitness of *M. discoidea*.

The origin of the populations affected the characters of the seeds. We observed that the soil treatment had a predominant effect on first life stages, whereas climate and population origin treatments (especially at the landscape scale) strongly influenced growth and reproduction of the plants (flowering and seed production).

We demonstrated contrasted development and survival patterns of *M. discoidea* under controlled conditions, proving that genetic differences exist between urban and rural populations of this alien plant species.

Keywords: Alien plant invasions, urbanisation, eco-evolution, global changes

## **Influence of abiotic factors on the growth and accumulation of ingenol 3-angelate in *Euphorbia peplus* grown in hydroponics**

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*Euphorbia peplus* is an annual herbaceous weed of the Euphorbiaceae family that synthesizes secondary metabolites including ingenol 3-angelate.

Ingenol-3-angelate is an irritating hydrophobic diterpenic ester that has topical anticancer activity and appears as a new topically applied drug against skin cancer.

These active compound have not yet been synthetically produced and can only be obtained directly from the plant. This is why it is necessary to deepen research to move to large-scale production.

After preliminary tests, it has been found that the *Euphorbia* develops well in hydroponic culture and in controlled environment rooms where one can easily vary the climatic conditions (LED, temperature, CO<sub>2</sub>, relative humidity...) that have a direct or indirect influence on the development of secondary metabolites. Hydroponic substrates are an alternative to soil, we will analyze their physical-technical characteristics of permeability and retention curve and assess advantages/disadvantages of hydroponics substrates compares to soil.

Our next goal will be to identify phenological stages of this plant by setting up decimal codes according to the BBCH scale and to develop optimal management strategies for the production cycle of this plant to optimize growth and biomass as well as the biosynthetic pathway leading to the production ingenol 3-angelate. In order to do that, tests will be carried out to choose the right growing medium and the best nutrient solution that promote the growth

of plant in hydroponics. The optimization of climatic factors will be carried out using the Box-Behnken model with a complete factorial plan to three factors (Temperature, Photoperiod, Photosynthetic Photon Flux Density), and at three levels (-1, 0, +1).

# MODELLING DISTRIBUTION OF TREE STEM CIRCUMFERENCE BY SPECIES USING LIDAR DATA IN MIXED DECIDUOUS TEMPERATE FORESTS

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The development of sustainable forest management depends on an accurate description of the forest resources. The required information concerns the stand structure (stem density, development stages, gaps, etc.), composition (tree species) and dynamic (growth, removals, and regeneration). However, large scale field measurements are time consuming and generally inconceivable.

New remote sensing technologies, in particular aerial LiDAR, raise new opportunities for large scale forest data collection. In particular, the Individual Tree Crown (ITC) method combines tree detection and crown segmentation to estimate tree height and crown attributes. Allometric models are then used to estimate girth, volume, biomass, etc. Yet, a frequent issue with ITC is the low detection rate of dominated trees (understory), especially in mixed irregular forest stands.

Our research aims to develop a new method using aerial LiDAR data to carry out a large scale forest inventory in a mixed uneven-aged forest of 22 Kha in the Ardenne ecoregion (southern Belgium). We are developing tools to estimate tree species and girth distributions by species in forest stands. This method provides thus a high resolution description of the forest that can be used to assess wood and carbon stocks, improve forest management plans and provide guidelines for policy makers.

**High resolution mapping of population change in breeding birds in Wallonia (Southern Belgium)**

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Patterns of fine-scale change in bird abundance across different landscapes may inform about the driving forces behind bird communities evolution. We compare km<sup>2</sup>-resolution map of breeding birds in Wallonia (Southern Belgium) at 10 years interval. The maps are based on repeated sampling transects conducted inside a km<sup>2</sup>-grid. Spatial modelling techniques were applied on these two dataset using environmental variables produced by the LifeWatch-WB Project. Variables are issued from pixel-based land cover classification of orthophoto mapping and satellite images, with a resolution of 2 meters and are available for the two periods corresponding to bird data. Others variables included in the model are topographic and soil attributes. For each bird species, spatial models built with data from the first period are projected with the value of the environmental variables for the more recent period, and vice-versa. Moreover, another method is to compare models built independently on the two periods. GAM (Generalized Additive Models) are used for modelling. These analyses allow to study if it is possible to predict the change in bird populations thanks to the data of changes of land cover.

# Flexible habitat use of an open-habitat bird species in a farmland-woodland landscape of southern Belgium

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Behavioural flexibility is the first way for organisms to respond to anthropogenic modifications of the environment. In the Belgian Ardenne, harvesting of plantation forests have created large clear-cut patches, while in the same time, production of Christmas trees has massively developed in farmland. These novel habitats were colonized by a number of bird species formerly associated to grassland in this landscape. However, as birds rely on environmental cues for habitat selection, their choice may be maladaptive if the cues become uncoupled from the underlying habitat quality. Therefore, in order to test if habitat selection is adaptive in this changing landscape, we compared the attractiveness and breeding quality of clear-cut patches, Christmas tree plantations and grassland for the European Stonechat *Saxicola torquatus*, used as a model species between 2014 and 2018.

Examination of the settlement pattern of territorial males indicated that stonechats preferentially settle in clear-cut patches, where their fitness (reproductive performances and survival) was not found to be higher. They produced there lower-quality offspring due to a gradual decrease of nestling body conditions during the breeding season, but we failed to demonstrate that this had a negative consequence on first-year survival probabilities and thus on parental fitness. As other parameters of reproductive performances, adult survival and first-year survival were similar between the three habitats, we concluded that clear-cut patches, Christmas tree plantations and grassland may provide breeding opportunities of similar quality to stonechats, although they strongly differ in terms of vegetation structure and management.

## **Modeling the reduction in sapling growth, density and diversity along a gradient of wild ungulate density.**

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Wild ungulates have determinant impacts on temperate forest regeneration. They causes shifts in species composition, alter forest structure and functioning. Their abundance has strongly increased for the last decades in the forests of the northern hemisphere. Although these effects have already been documented for some particular study cases, a deeper and more general understanding of these effects is needed across varying environmental conditions, and particularly across varying densities of wild ungulates.

In 2016, a vast network of 970 enclosure-exclosure experiments was set up in southern Belgium. Each of the 970 plots was established in forest areas where natural generation was present or expected. The plots were composed of one fenced subplot of 6 m<sup>2</sup> (control) and two unfenced square subplots of 6 m<sup>2</sup> at a 4 meter between centers distance. The heights of the highest seedlings, the number of seedlings of each species, the herbaceous and shrub covers were measured at the installation, and in 2017 and 2018. That network covers a region of 3000 km<sup>2</sup> and a wide gradient of abundance of red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*).

To model the effects of red deer and wild boar on forest regeneration, we fitted non-linear models to predict regeneration height growth, density and diversity in the unfenced plots in response to observations, carried out in the adjacent fenced plot: red deer abundance, wild boar abundance and different environmental conditions as altitude, soil and basal area. A relationship was then established between the severity of ungulates impacts on forest ecosystem and the density of ungulates in different environmental conditions. Such understanding will be particularly helpful to design adequate forest and wild game management guidelines.

## **Why is birch an interesting species for forestry and forest-based industry sector within the changing climatic and socio-economic context of Western Europe?**

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In Western Europe, most of the few commercial tree species struggle with climate change or health problems. This highlights the need to adapt forest management and consider new tree species. At the same time, people are demanding for practices that are more in line with biodiversity and natural cycles. Birch (*Betula pendula* Roth and *B. pubescens* Ehrh.) is an indigenous pioneer tree species that naturally and abundantly regenerates in all kind of soil conditions throughout Western Europe. However, it has been neglected by forest managers and forest-based industry, probably due to the poor quality of the resource. Nevertheless, birch provides many ecosystem services that go along with forest sustainability. In addition, with proper silvicultural treatment, birch produces high value timber with interesting aesthetic and mechanical properties, which can be used in furniture and in construction. Our thinning experiments show a huge benefit in radial growth when birch crown is released from competition since a very early age. This makes possible to produce logs of 150 – 180 cm in circumference within 50 years. Improved knowledge about birch development needs and analyze of the strengths, weaknesses, opportunities, and threats of the use of birch for forestry and forest-based industry sector within the changing climatic and socio-economic context of Western Europe, permit to provide practical answers to practitioners, industry and decision makers.

## Abstract

### **Analysis of the morphological diversity of elite Shea trees (*Vitellaria paradoxa*, C. Gaertn.) identified in the Bagoué and Tchologo counties, Côte d'Ivoire.**

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Shea tree (*Vitellaria paradoxa* C. Gaertn) is one of the most important economically and socially important tree species in the Sudano-Sahelian region and it is used in cooking, cosmetics, chocolate making and traditional medicine. It is widely distributed in the northern Côte d'Ivoire. However, it is the object of many pressures such as demography, bushfires, and misuse of trees which cause genetic erosion of the species. The knowledge and understanding of the genetic variation of elite shea tree genotypes identified in farmers' land is essential for conservation and improvement.

The aim of this study was to determine the relevant agro-morphological traits of elite shea tree in Côte d'Ivoire.

It was carried out on 220 shea trees randomly selected from a population of 639 elite shea trees in the Bagoué and Tchologo counties, northern Côte d'Ivoire. We assessed phenotypic variability based on 11 qualitative and 10 quantitative traits.

The results showed significant variability for most of the characteristics studied. Thus Shannon diversity index in the order of 0.98; 0.97; 0.95; 0.89 and 0.85 were found respectively for Shaft carrying, crown's shape, branching, blade apex shape and nut's shape. Results also showed high variation of number of fruit per tree (CV=39.9%), stem circumference (CV=30.71%), nut weight (CV=28.51%) and petiole length (CV=24.41%). Correlation was positively strong between nut yield and stem circumference ( $r=0.772$ ;  $p<0.001$ ), leaf length and leaf width ( $r=0.568$ ;  $p<0.001$ ), nut weight and nut width ( $r=0.808$ ;  $p<0.001$ ) and nut length and nut weight ( $r=0.580$ ;  $p<0.001$ ). The stem circumference was the only one quantitative morphological variable that structured elite shea trees into three groups. A combination of both quantitative and qualitative traits had identified 10 relevant descriptors that could describe efficiently elite shea trees in Côte d'Ivoire. Those morphological traits include 5 qualitative (nut color, nut shape, crown shape, young leaf color and limb shape) and 5 quantitative traits (stem circumference, nut length, nut weight, limb length and width). In order to complete the study of shea tree genetic diversity in Côte d'Ivoire, molecular markers will be used.

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## **Predicting forest site characteristics from floristics ; an automated approach for forest management in the Walloon Region.**

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Forest tree and plant species prosper in the specific ecological conditions of their ecological niche. Together, the proper knowledge of the ecological niche of tree species and the proper description of a Forest site form a keystone that ensures a good match between forest trees and their environment. In Wallonia, an original toolbox is in use since 1991, made up of an extensive documentation about tree species ecological requirements and a comprehensive methodology of forest site characterization. Gathered under the denomination of "forest tree autecology tool", this toolbox has been recently reviewed and updated by a scientific consortium and is now available online (<https://www.fichierecologique.be/>). The objective of this toolbox is to determine which tree species fits the best a particular forest site. It is based on the location of the site and of the ecological niche of the tree species into a 3 axis matrix "ecogram" (climatic zone, nutrient and moisture level) summarizing soil and topographic information, issued from direct observations or cartographic data, thanks to dichotomic keys.

Along with the abiotic approach that helps to position the forest site in the ecogram, a floristic approach has also been used independently for years. Thange (1969) has settled the foundations of the socio-ecological groups, which are defined as a set of forest plant species who share the same ecological niche. These socio-ecological groups are commonly used in southern Belgium to describe forest sites. But still, the determination of the nutrient and moisture levels of a forest site based on its species composition is a complex task which requires a high level of expertise. Here, we present an automated approach which aims at determining nutrient and moisture levels of any sites from the plant species sampled. We believe that an increased level of automatization will promote the use of these methodology of forest site identification, tested for long but requiring detailed expertise.

Numerous floristic samples were compiled over years and resulted in a comprehensive collection of 2074 forest site observations, which grasp all the nutrient and moisture gradients of Wallonia. Determination of the nutrient and moisture levels for each site has been achieved by the use of the abiotic dichotomic keys from the "forest tree autecology tool". An automated classifier algorithm (random forest) was trained with these data in order to predict the nutrient and moisture levels of any new sites in Wallonia from the presence or absence of every indicator plant species of the Wallonian ecological groups [n=260 species tested]. Results suit well our expectations and this classifier has been integrated into a web application dedicated to forest managers.

# Calibrating individual-based models using Approximate Bayesian Computation

L. De Visscher <sup>1</sup>, J.M. Baetens <sup>1</sup>, B. De Baets <sup>1</sup>

<sup>1</sup> Ghent University, Belgium

The rise in computational power has allowed researchers to develop computer models that simulate basic demographic processes and interactions of many individual organisms out of which complex and unexpected behaviour can emerge. These so-called individual-based models (IBMs) have become increasingly popular in ecology, as they have the potential to bridge the gap between the behaviour of individuals and populations in a mechanistic way. However, while providing a flexible and easy-to-interpret means of simulating ecological systems, IBMs often depend on many model parameters. Moreover, as most statistical methods for parameter estimation cannot readily be used for IBMs, their calibration is challenging.

A family of statistical methods named Approximate Bayesian Computation (ABC) has recently been proposed as a tool for the calibration of IBMs. In this work, we explore to what extent ABC methods can be used to infer the parameters in a benchmark individual-based competition model involving three species. ABC seems well-suited to tackle this problem, returning realistic parameter distributions, which in turn can provide useful insights in the model behaviour and pave the way for calibration using real observation data.

## **Edible caterpillars in the savannah around the Luki Biosphere Reserve in the Democratic Republic of Congo**

Ernestine Lonpi Tipi<sup>1</sup>, Joseph Lumande Kasali<sup>2</sup>, Damase Khasa<sup>3</sup>

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The Luki Biosphere Reserve (RBL) in the Democratic Republic of Congo is the last refuge of the Mayumbe forest. Population around this reserve lives mainly from the exploitation of forest resources.

Around the RBL, most of the vegetation is savannah. These savannahs are subjected to annual bush fires, with a negative impact on the fertility of soils which, by their nature, are already poorly fertile.

To meet the conservation needs of this reserve's forest resources, several actions have been initiated by international and national NGO, with the aim of offering residents, relatively fertile land for agricultural production and preserving ecosystems in the RBL.

Savannahs have been protected against bush fires in a natural forest regeneration process. Edible caterpillars are among the goods collected from regenerated savannahs, forest galleries, shrub-to-tree savannahs and fallow land in the villages surrounding the RBL. These caterpillars are an important source of animal protein and income for rural and urban households. However, households complain about the random and episodic presence of edible caterpillars which is probably related to threats on their host plants.

To date, very few studies have been carried out on edible caterpillars in a forest resource management context at RBL. This study, which is still in its infancy, is part of a PhD research and would like to contribute to a better knowledge of the edible caterpillar species of the savannahs around the RBL, to determine the factors influencing their presence and abundance on the host plants, with a view to an analysis of the corresponding value chain and the conditions for its domestication.

## **The impact of information-transfer related to soil biodiversity on Flemish citizens' preferences for forest management**

Iris Vanermen<sup>1</sup>, Kris Verheyen<sup>2</sup>, Bart Muys<sup>1</sup>, Liesbet Vranken<sup>1</sup>

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Despite playing a crucial role in the delivery of forest ecosystem services, soil biodiversity is under increasing pressure, partly caused by high intensity forest management. While managers are recently increasing attention for soils, they are often obstructed by public opinion, that is highly ignorant of soil biodiversity. Insights in public preferences of forest management and methods to steer these preferences are hence needed to facilitate adoption of sustainable management practices. Nevertheless, such insights are largely lacking. This study wants to address this gap through a discrete choice experiment with a representative sample of 299 Flemish citizens. To assess the effect of information on preferences, the discrete choice experiment consisted of two rounds with an informative video on soil biodiversity in between. Results showed that, in general, Flemish citizens had significant positive preferences for the sustainable levels of the management choices both before and after the information transfer, except for the highest level of old trees and dead wood. Preferences for this level were insignificant before and became positive significant after the information transfer. Moreover, preferences for the three pure management attributes generally became more pronounced after the information transfer, while a decrease in effect size was found for the monetary attribute. These results indicate that Flemish citizens were mostly unaware of the crucial role of old trees and dead wood prior to the information. Through information transfer support for sustainable management practices could however be strengthened. Ultimately, this would benefit soil biodiversity and protect future delivery of forest ecosystem services.

## Session 3 (ES)

# Green chemistry & bio-products

15h30 -16h30

### Chairman David Cannella – ULB

Seasonal effect on the chemical composition of essential oils hydrodistilled from *Zanthoxylum leprieurii* Guill. & Perr. and on their biological activities

Tanoh Amenan Evelyne (ULiège)

Lignin-first fractionation of lignocellulosic biomass from agricultural residues

Filippo Brienza (UCLouvain)

Bio-based ortho-methoxy groups ameliorate classic bisphenol chemistry

Steven-Friso Koelewijn (KULeuven)

Characterization of pyomelanin pigment produced by *Y. lipolytica* and its application in green synthesis of gold nanoparticles

Imen Ben Tahar (ULiège)

Flash presentations

## **Chemical composition change in cellulosic biomasses after their torrefaction**

Bruno Godin, Thibaut Masy, Olivier Hecq, Richard Agneessens, Jérôme Delcarte  
*Centre wallon de Recherches agronomiques - Belgium*

The outlook of torrefying cellulosic biomasses is to be able to produce a renewable fuel under the form of pellets comparable to mineral coal, both in terms of energy density and physicochemical characteristics. In addition, it aims to replace fossil coal with a renewable fuel.

The objective of our study is to assess the content change of the main chemical components (Lignin, Cellulose, Hemicelluloses, Solubles Sugars, Starch, Proteins and Mineral compounds) induced by torrefaction (at 270°C until a 25% loss of dry weight) in cellulosic biomasses (Bamboo, Spruce, Tall fescue, Corn and Sorghum). In addition, we assessed the suitability of the Van Soest method that is usually used to determine the content of lignin, cellulose and hemicelluloses in forage biomasses to be used with torrefied biomasses.

Our results show that torrefaction enables to have biomasses with a more standardized chemical composition and lower water content. A drawback of torrefaction is to concentrate the content of mineral compounds. The observed changes in chemical composition suggest that cellulosic biomasses use for torrefaction should have high contents of lignin, cellulose and hemicelluloses and a low content of mineral compounds.

In contrary to cellulosic biomasses, it is necessary in torrefied biomasses to determine the protein content in the NDF, ADF and ADL Van Soest fractions to subtract it from each respective fraction to avoid biased Van Soest values of the lignin, cellulose and hemicelluloses contents. This is necessary for a higher degree of accuracy of the total mass balance of torrefied biomasses.

## Seasonal effect on the chemical composition of essential oils hydrodistilled from *Zanthoxylum leprieurii* Guill. & Perr. and on their biological activities

Evelyne A. Tanoh <sup>1,2\*</sup>, Blanchard G. Boue <sup>1</sup>, Fatimata Nea <sup>1,2</sup>, Henri Martin<sup>2</sup>, Manon Genva <sup>2</sup>, Allison Ledoux <sup>3</sup>, Felix Z. Tonzibo <sup>1</sup> and Marie-Laure Fauconnier<sup>2</sup>

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3. CIRM, Laboratory of pharmacognosy, University of Liège, avenue hippocrate 15, 4000 Liège, Belgium

This study focused on evaluating the seasonal effect on the chemical composition and on the biological activities of essential oils hydrodistilled from leaves, trunk bark and fruits of *Zanthoxylum leprieurii* (*Z. leprieurii*) over the months, describing the climatic conditions of specific seasons of Côte d'Ivoire. *Z. leprieurii* is a plant commonly used in traditional medicine. Besides, some of its metabolites have already shown antioxidant, antimicrobial, anticancer, cytotoxic, schistosomidal and antibacterial properties <sup>1,2</sup>.

Essential oils were hydrodistilled from organs with a Clevenger-type apparatus and analyzed by gas chromatography-mass spectrometry (GC/MS). Essential oil of leaves were dominated by sesquiterpene and methylketones, such as tridecan-2-one, (*E*)- $\beta$ -ocimene,  $\beta$ -caryophyllene, dendrolasin, undecane-2-one and thymol. Fruits essential oils were characterized by monoterpenes with  $\beta$ -myrcene, citronellol, geranial and methyl nerate. Essential oils of trunk bark were commanded by methylketones, as the main compounds were tridecan-2 one,  $\beta$ -caryophyllene,  $\alpha$ -humulene, tridecan-2-ol and (*E,E*)-farnesol. .

Results showed that the seasonal effect does not statistically impact the chemical composition of essential oils hydrodistilled from the different organs of the plant. Besides, the essential oils investigated in this work have exhibited significant antioxidant, anti-inflammatory, insecticidal and moderate anti-plasmodial activities. Those activities were related to some compounds identified in the essential oils. In conclusion, this investigation confirmed the high potential of *Z. leprieurii* for a use in traditional medicine.

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# Injection of essential oils in xylem of apple tree for management of sap-sucking pest in orchards

Pierre-Yves Werrie, Chemistry of natural molecules laboratory, University of Liège, Belgium

Guillaume Jean Le Goff, Earth and life institute, biodiversity research centre, Université Catholique de Louvain, Belgium

Prof. Thierry Hance, Earth and life institute, biodiversity research centre, Université Catholique de Louvain, Belgium

Prof. Marie-Laure Fauconnier, Chemistry of natural molecules laboratory, University of Liège, Belgium

Apple aphid (*Disaphys plantaginea*) is responsible for significant yield declines in apple orchards through sap-sucking activities. To manage them, farmers have so far used a wide range of phytosanitary products, sometimes harmful to both environment and human health. Therefore this research aims to develop alternative product based on essential oils injected into the tree vascular system (xylem).

An experimental design in climatic chamber was establish using Jonagold M9 2Y trees, Baxter bags and needles to inject emulsion of *Cinnamomum cassia*, *Mentha spicata* and *Eugenia caryophyllus* essential oils.

To demonstrate the biological activity of these treatments mortality curves were establish using 3 days old larvea of *Dysaphis* in clip cage.

The phytotoxicity was assessed through eco-physiological measures of chlorophyll fluorescence and photosynthetic activity.

The content kinetics of the major volatile organic compounds (VOCs) of the oils as well as their impact on the rest of the VOCs profile (both contained and emitted by leaves) was performed by thermal desorption and dynamic headspace gas chromatography coupled to mass spectrometry (TDU-GC-MS and DHS-GC-MS).

The results demonstrate 80% death rates after 3days treatments as well as presence of oils compounds within the leaves without significantly impacting the leaf vitality parameters compared to blank treatments. Those results demonstrate the case for essential oil in agricultural practises.

## Seasonal variation, yield composition and biological activities of essential oils from *Lantana camara* grown in Côte d'Ivoire.

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<sup>d</sup>University of Corse-CNRS, UMR 6134 SPE, Route des Sanguinaires, F-20000 Ajaccio

*Lantana camara* is a thorny shrub that goes from 2 to 5 m high. Its ripe fruits are blackish and its flowers are in various colors<sup>1</sup>. It is used in traditional medicine to treat several diseases and has numerous properties that were chemically described such as antioxidant<sup>2</sup>, antibacterial<sup>3</sup> and cytotoxic<sup>3</sup> activities.

The aim of this work was to study the variations in *L. camara* essential oils composition during the vegetative cycle. Leaves, flowers, fruits and stems of *L. camara* were harvested in Bingerville (East Côte d'Ivoire) each month from June 2015 to June 2017. The essential oil was produced by hydrodistillation and then analyzed by GC-MS and RMN. The antioxidant, anti-inflammatory and insecticidal activities of leaf and flowers essential oils were also determined.

Results showed that essential oils hydrodistilled from *L. camara* are dominated by sesquiterpenes such as  $\beta$ -caryophyllene and  $\alpha$ -humulene. Some monoterpenes such as thymol, sabinene and  $\alpha$ -pinene were also present. The oil yield was high during the flowering and fruiting period. According to the chemical composition of *L. camara*'s oil, there were no significant differences between both harvesting periods defined but there were significant differences from one organ to another. However, within one organ the chemical composition varies under the vegetative cycle. It was found that the proportion of thymol was higher during flowering and fruiting months. The essential oil of stems, flowers, and fruits were more concentrated in thymol than leaf's oil. E- $\beta$ -caryophyllene and  $\alpha$ -humulene were found in all essential oils. However, their quantity and the thymol content were strictly inverted throughout the harvest period or vegetative cycle. In addition, the essential oil extracted from leaves and flowers of *L. camara* showed good antioxidant, anti-inflammatory and insecticidal activities.

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## Designing natural fibres for biocomposites

Sophie Morin<sup>1</sup>, Aurore Richel<sup>1</sup>

1. Laboratory of Biomass and Green Technologies, Gembloux Agro-bio Tech University of Liege,  
Gembloux, Belgium

**Tuneable natural vegetal fibres were produced to be incorporated in plastic matrices in accordance to the green chemistry principles. The development of versatile tools is an immediate solution to the actual industrial plastic issues.**

Plastics are a worldwide societal and environmental issue. Industries are expected to find quick alternatives of plethora daily-products while guarantying the same quality.

Bioplastics (ie bio-sourced and/or biodegradable) already exist in the market but are either economically non-viable or non-usable for many applications. Alternative to these materials consists in combining already-in-use plastic matrices with sustainable bio-resources. Natural fibres are a highly abundant resource suitable to create sustainable materials.

Incorporating natural fibres in polymer matrices is challenging as both components tend to repel each other. Industrial fibres specifications focus on multiple fibres parameters including fibres aesthetic, composition, cost and safety. The fibre chemico-enzymatic engineering is explored to modify and improve the fibres properties. Fibres dispersity in the polymer was enhanced, mechanical resistance and fibres coloration modified after enzymatic treatment. Chemical treatment affected fibres composition, coloration, wettability and crystallinity.

The developed methods are versatile tools to design fibres in accordance with each final product specification.

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## **Reactivity of glucan and xylan in brewer's spent grain : Comparing of dilute alkaline versus liquid hot water pretreatment**

Bruno Godin<sup>1</sup>, Grégoire Henry<sup>2</sup>, Thibaut Masy<sup>1</sup>, Thomas Nicolay<sup>2</sup>, Patrick A. Gerin<sup>2</sup>, Jérôme Delcarte<sup>1</sup>

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To be able to produce biofuels from cellulosic biomass in an economically efficient way, it is essential to make it less recalcitrant to enzymatic hydrolysis. This can be achieved by pretreating the biomass to get its glucan (cellulose) and xylan (hemicelluloses) more reactive. A laboratory-scale pressure reactor (Parr 4540) has been used to increase the reactivity of glucan and xylan in brewer's spent grain (4% of dry solid loading) after dilute alkaline (120 minutes 80°C using KOH 0.1M) or liquid hot water pretreatment (20 minutes at 140°C or 220°C). The glucan, xylan and Klason lignin of the solid and liquid fractions of these assays were determined by the standard NREL (or equivalent) procedures. The corresponding gain in biodigestibility were assessed by the biomethane potential of the pretreated biomass. These experiments showed that dilute alkaline pretreatment at low severity enables an interesting reactivity of brewers's spent grain in comparison to liquid hot water pretreatment which is a pretreatment with a higher severity.

## **From Belgian agro-industrial residues toward green chemistry; Study of brewer's spent grain continuous fermentation conditions by a mixed microbial community**

Grégoire Henry<sup>1</sup>, Florent Awedem Wobiwo<sup>1</sup>, Maxime Velings<sup>1</sup>, Patrick Gerin<sup>1</sup>  
1 UCLouvain, Belgium

Brewer's spent grain (BSG) is roughly 85% of the total by-product generated from beer brewing. It is usually used as animal feed. BSG contains proteins and carbohydrates, including residual starch and (hemi)celluloses from the hull. It is therefore usable as carbon and/or nutrient source in biotechnological processes, including in mixed culture fermentation processes. Our aim is to identify the best acidogenic fermentation conditions in order to produce a mixture of medium chain carboxylates (MCFAs). The targeted MCCAs, n-caproate and n-caprylate, are valuable chemicals, which can be used as intermediate molecules for green chemistry. In our preliminary approach, the mixed culture fermentation is performed with BSG as raw substrate, wastewater sludge as inoculum, H<sub>2</sub> as electron donor and exogenous addition of ethanol. The influence of the Organic Loading Rate (ORL), Hydraulic Retention Time (HRT), H<sub>2</sub> and ethanol exogenous addition on the carboxylates production rates will be presented.

## Exploration of exopolysaccharide production by *Cyanothece sp.* PCC 7822

**Camille Van Camp and Ruddy Wattiez**

**Proteomic and Microbiology laboratory, University of Mons, Belgium**

The present research is part of the Algotech project based on the production of high-added value products by Microalgae/Cyanobacteria related to a circular economy. In this context, we investigate exopolysaccharide (EPS) production by the diazotrophic strain *Cyanothece sp.* PCC 7822. Cyanobacterial EPS have singular properties and applications in cosmetics, medicine or food industries. This study focuses on the impact of C/N ratio by testing various nitrogen sources ( $\text{NaNO}_3$ , atmospheric nitrogen,  $\text{NH}_4\text{Cl}$  and urea at different concentrations) on bacterium metabolism and EPS production. Regardless of the nitrogen concentration tested (1 mM, 2.5 mM and 17 mM in term of N),  $\text{NaNO}_3$  induces the best growth of the strain. A reduction of nitrogen sources concentration impacts growth negatively but EPS production positively. Consequently, the EPS production reaches around 15% of dry biomass at 1 mM for all combined nitrogen sources. Surprisingly, maximum EPS accumulation obtained is risen 50% of the dry biomass in atmospheric  $\text{N}_2$  condition. In addition, optical observation of EPS by an alcian blue staining highlights variation of EPS configuration according to the culture condition. These uncommon results could be explained by a low nitrogenase efficiency inducing an N-limiting condition even if the strain highlights a diazotrophic metabolism. Further analyses concerning its metabolism and EPS composition in addition with potential industrial application will be of particular interest.

## **Lignin-first fractionation of lignocellulosic biomass from agricultural residues**

Filippo Brienza, Damien Debecker, Iwona Cybulska

Université catholique de Louvain, Belgium

Lignin monomers and short-chain oligomers, as well as purified cellulose and hemicellulose fractions, represent extremely valuable chemicals for green chemistry applications, including biofuel production, polymers manufacturing and pharmaceutical applications. Currently, no method is available that would offer a means to achieve an efficient, industrially feasible, process for the fractionation of lignocellulose into such interesting intermediates. This research is targeting the controlled lignin extraction and depolymerization from agricultural residues (e.g. wheat straw, corn stover) through catalytic hydrogenolysis and hydrogenation during the "solvolysis" process, which yields lignin oligomers and phenolic monomers (guaiacyl, syringyl and coumaryl alcohol derivatives). The influence of operating conditions on delignification, lignin depolymerization and carbohydrate retention has been investigated. Catalysts based on transition metals (e.g. Ni) have been studied with the goal of replacing the more expensive precious metal catalysts employed in the current research. In parallel, the possibility of eliminating the hydrogen gas used in the process, by substituting it with hydrogen donor solvents, has been inspected. The intermediates produced within the process (including the lignin oil and the carbohydrate fraction) have been characterized to assess its performance, as well as to evaluate the most favorable routes for further transformation and valorization of the obtained products. Process optimization has been performed aimed at reducing the process severity, while maximizing the yield of lignin mono/oligomers and recovered carbohydrates, thus targeting future industrial implementation.

## Production of poly(HB-co-HHx) by *Rhodospirillum rubrum* using hexanoate as carbon source

Paloma Cabecas Segura<sup>1</sup>, Ruddy Wattiez<sup>1</sup>, Baptiste Leroy<sup>1</sup>

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Polyhydroxyalkanoates (PHA) are bio-sourced, and biodegradable polyesters used as internal carbon storage by bacteria. These polymers represent an environmentally friendly alternative to petroleum-based plastics for a broad range of applications from packaging to biomedical devices. As PHB is highly crystalline and brittle material, some copolymer containing hydroxybutyrate (HB) and hydroxyhexanoate (HHx) monomers are rather chosen for biomedical applications. Among the few bacteria known to synthesized poly(HB-co-HHx), *Rhodospirillum rubrum* has been chosen for its ability to produce this polymer using hexanoic acid, as carbon sources. In this study, the production of polymer is not driving by a nutrient unbalance in the medium. Here PHA synthesis hypothetically serves as an electron sink because of the consumption of NADPH during the process. Polymer production, composition and accumulation were investigated in those conditions. To increase the proportion of HHx, a better understanding of the assimilation pathway of hexanoate in *Rs.rubrum* is needed. Proteomic analysis and mutant fitness essay have been performed and highlighted that its assimilation occurred through  $\beta$ -oxidation and, two main anaplerotic pathways: the ethylmalonyl-CoA and the methylbutanoyl-CoA pathway. Based on this knowledge, four methods have been tested to improve both monomer incorporation and accumulation of PHA, leading to the obtention of an increased accumulation and HHx content.

## MOF screening for fructose conversion into 5-hydroxymethylfurfural: an experimental study

Noor Aljammal<sup>1,2,#</sup>, Alexandra Lenssens<sup>1,3,#</sup>, An Verberckmoes<sup>3</sup>, Joris W. Thybaut<sup>4</sup>, Francis Verpoort<sup>1,5</sup>, Philippe M. Heynderickx<sup>1,2,\*</sup>

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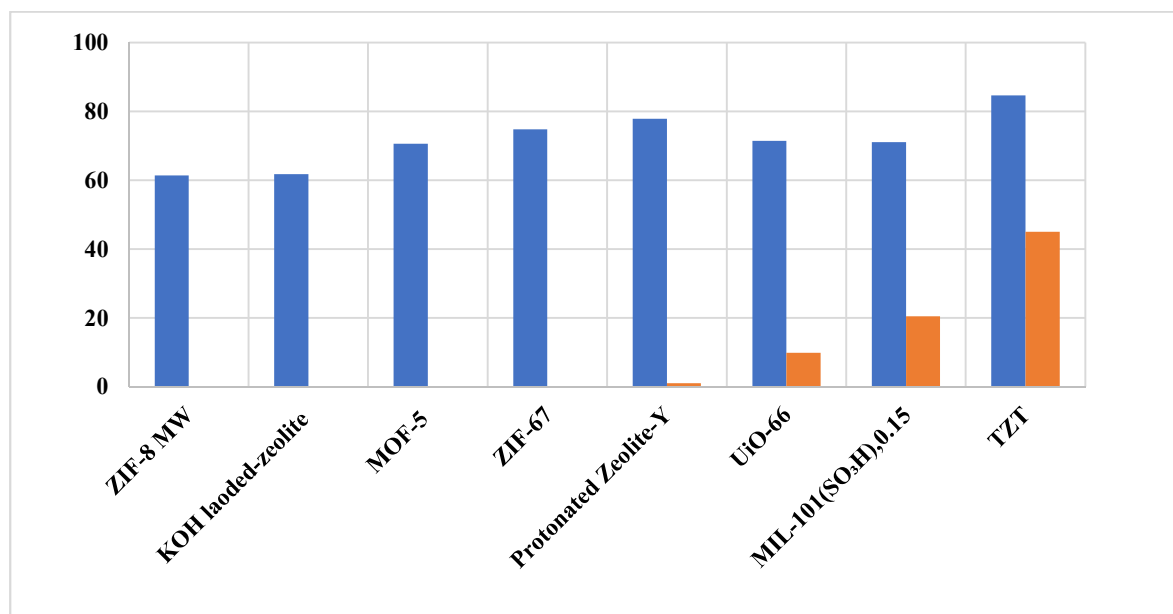
The dramatic concerns about global warming and the limitation of fossil fuel reserves have triggered the need to develop renewable liquid fuels that are sustainable and environmentally friendly [1].

In this work, the specific focus is on the conversion of fructose as biomass source into essential platform chemicals, more particularly 5-hydroxymethylfurfural (5-HMF). The major motivation is that 5-HMF is one of the essential platform chemicals, as it embraces a key status between the production of polymer precursors, liquid biofuels, and essential intermediates for the production of fine chemicals [2]. Novel catalysts are expected to play a pivotal role in the sustainable production of biomass-derived products. However, high costs because of low product selectivity and stability is a significant challenge impeding a broad practical implementation and commercialization of catalyst-based biomass conversion. In this context, several MOFs-based catalysts were synthesized and tested in fructose dehydration into 5-HMF via microwave-assisted reactor using dimethylsulfoxide/acetone (DMSO/Ac), water, and biphasic aqueous solvents like  $\gamma$ -valerolactone /water (GVL/w).

Commercial and modified zeolites were used as benchmark catalysts for catalytic performance comparison. The one-pot reaction was carried out in a batch system using 100 g fructose, 30 mg catalyst at 150°C temperature, and 5 min reaction time.

The catalytic performance of each MOF was evaluated according to its surface area, pore size, defects, and the number of unsaturated sites. Fig. 1 shows satisfactory fructose conversion levels for all studied catalysts, but the yield of 5-HMF is negligible for most of the catalysts. As a catalyst, the ZIFs materials generated excellent fructose conversion. Interestingly, greater quantities of side products such as formic and lactic acids were produced. The lack of strong Brønsted acidity in ZIFs materials resulted in poor HMF yields. The higher yield was obtained over MIL-101 SO<sub>3</sub>H. This result is attributed to the fact that sulphonated MOF-MIL-101(Cr)-SO<sub>3</sub>H possesses high hydrothermal stability, high surface area, and suitable pore sizes, and it contained both a strong Lewis acid site from Cr<sup>3+</sup> and a Brønsted acid site from grafted –SO<sub>3</sub>H groups. This combination would afford significant enhancement of the MOF performance as a heterogeneous catalyst in the carbohydrate dehydration [3]. Yet, the

performance of modified zeolite, TZT, outperforms all the studied MOFs, due to its high acidity and stability.



**Figure 1: Comparison of catalytic results for the reported conditions over various catalysts. (■) Fructose conversion (%), (■) HMF yield (%).**

Future work will consist of more intense screening on the sulphonated MIL-type catalysts, as they currently exhibit the best conversion and selectivity towards HMF. Also, modification on the parent MOF, as an increasing sulphur grafting rate, can be subject to further investigation. Ultimately, the experimental results will be used in a kinetic study in order to optimize reaction conditions and catalyst properties.

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## Influence of reaction conditions on produced hydrochar based on waste seaweed via hydrothermal carbonization

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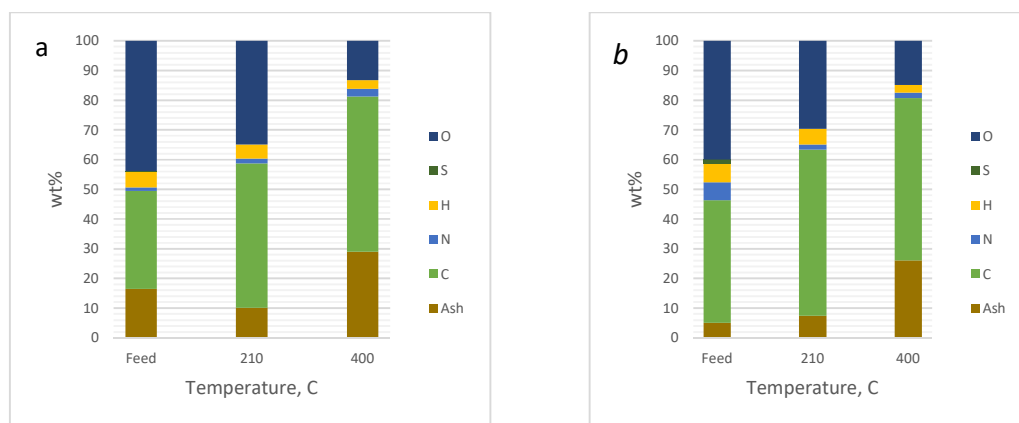
On the coastline of South Korea, yearly 177,000 metric tons of seaweed are washing up. While this causes odor nuisance it also is not appealing for touristic purposes. Cleaning the beaches from this seaweed is expensive [1]. Therefore, economic valorization of the waste seaweed has to be found.

It is reported that seaweed is a good feedstock to produce hydrochar, because of its availability and high nutrient content [2, 3]. This hydrochar can be applied as an adsorbent for pollutants or as soil amendment to increase fertility and carbon sequestration [4, 5].

Hydrochar is produced as a slurry (a two-phase mixture of solid and liquid) via so-called ‘hydrothermal carbonization’ (HTC). This hydrothermal process takes place in the presence of water at high temperature, up to 400°C, and corresponding pressures up to 20 MPa [5]. Among the main advantages of this relatively novel feedstock is that fresh seaweed contains around 90% water, which is ideal for HTC conversion and no feedstock drying is required as would otherwise be the case in the more traditional carbonization or slow pyrolysis. In this work, for the experiment’s sake, all material was dried prior to further analysis or treatment.

HTC was carried out in an autoclave reactor for two different types of seaweed, being *Sargassum* and *Ulva pertusa*, collected in the Busan coastal area of June 2019. Reaction temperatures ranged from 180°C to 400°C with different water to dry material ratios (1:1 to 12:1, w:w) and the reactor was held at the final temperature for a residence time of 2 or 4 hr.

Temperature had a notable effect on the composition of hydrochar, see Figure 1. By increasing the HTC temperature, hydrolysis and dehydration reactions take place and the carbon content in the hydrochar subsequently increased. More specifically, the sharp decrease is most probably due to the conversion of glucose, coming from cellulose in the original seaweed, into other water soluble degradation products, such as levulinic acid, dihydroxyacetone and formic acid. A significant factor for the decrease in HTC yield can be ascribed to the fact that the majority of the oxygen is transferred to the liquid-phase and it is incorporated into dissolved organics [6].



**Figure 1: Effect of HTC temperature on elemental composition (dry basis), a) *Ulva pertusa* b) *Sargassum*.**

The effects of biomass residence time and water to feedstock ratio on the HTC yield, and hydrochar composition were explored starting from *Ulva pertusa* and *Sargassum*. The HTC yields, together with the corresponding elemental composition, were analyzed. In addition, specific surface area and pore size was obtained: as expected, increasing residence time and temperature resulted in an increase of the porosity and surface area, while the amount of HTC char decreased.

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## Innovative biosourced matrices to encapsulate essential oils with a controlled release

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The long-term harmful effects of chemical pesticides are one of the major controversies these days. Following the objective of reducing the use of these products without decreasing crop yield, essential oils (EOs) are a prime candidate for biocontrol. However, high volatility of EOs begets a challenge: increasing the duration of efficient activity of EOs.

In this work, an innovative green matrix for essential oil retention is proposed. Indeed, glycerol carbonate surface-modified dendrimers (GDs) have shown their ability to encapsulate some metallic complexes and organic compounds (Balieu S. *et al.*, 2013; Menot B. *et al.*, 2015). In addition, two others new types of glycerol based dendrimer has been use: “glyceroladendrimer” and “glyceroclickdendrimer”. As a consequence; the final goal is to produce an efficient slow release biosourced herbicide based on a glycerodendrimer - essential oil combination.

*Cymbopogon winterianus* Jowitt and *Cinnamomum verum* Presl essential oils have been chosen for their herbicide properties. The total retention rate in solution was determined by dynamic headspace gas chromatography coupled with mass spectrometry (Kfoury M. *et al.*, 2015). Results show that dendrimers encapsulate essential oils with some efficiency.

Furthermore, interactions between GDs and EOs were studied by nuclear magnetic resonance spectrometry and infrared spectrometry. In parallel, efficiency of created products has been controlled by analyzing the inhibition of *Arabidopsis thaliana* seed germination.

The poster will focus on optimization of the EOs retention by biobased dendrimers, the interactions mechanisms and their efficiency as biosourced herbicide.

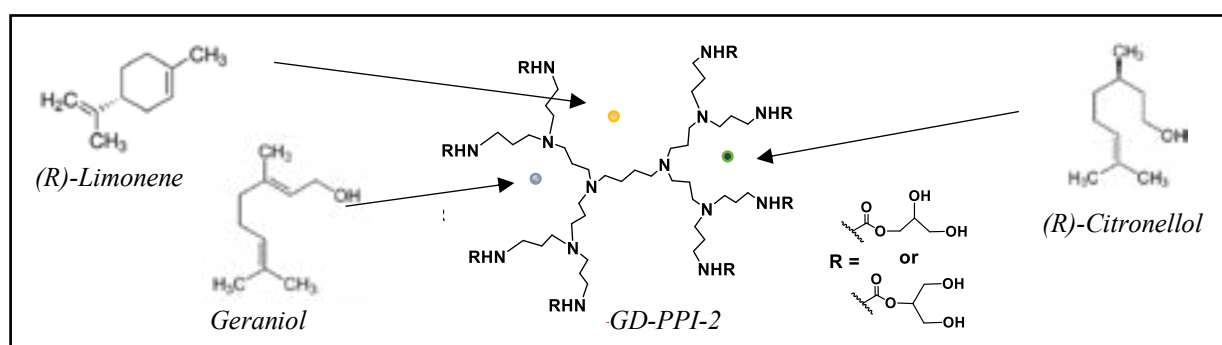


Figure 1. Encapsulation of *Cymbopogon winterianus* Jowitt major compounds (citronellol, geraniol and limonene) in the second generation of glycerodendrimer poly(propylenimine) (GD-PPI-2).

**Key-words: dendrimer – essential oil – biosourced herbicide - encapsulation**

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## BIO-BASED ORTHO-METHOXY GROUPS TO AMELIORATE CLASSIC BISPHENOL CHEMISTRY

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### ABSTRACT

Bisguaiacol F (BGF), a potentially safer and renewable bisphenol A (BPA) replacement made from lignin-derivable vanillyl alcohol (*p*-VA), is a promising building block for future aromatic biopolymers. Unfortunately, like BPA synthesis, this electrophilic condensation reaction is also prone to regioselectivity issues, giving rise to *m,p'*- and *o,p'*-BGF byproducts.<sup>1</sup> In this work,<sup>2</sup> the hitherto unconsidered influence of *m,p'*-BGF, *viz.* the main isomeric byproduct of *p,p'*-BGF synthesis, on the physicochemical properties of poly(BGF carbonate) (BGF-PC) was systematically investigated by random copolymerisation with different fractions of pure *m,p'*-BGF (25, 50 and 75 wt%). To do so, the *m,p'*-isomer was made in unparalleled regioselectivity (72%) by alkylation condensation of isovanillyl alcohol (*m*-VA) with guaiacol. Surprisingly, no isomeric scrambling due to acid-catalysed isomerisation was encountered for pure BGF isomers, which facilitates their synthesis in contrast to petrochemical bisphenol F (BPF). Furthermore, to ensure safer chemical design, an *in vitro* human oestrogen receptor  $\alpha$  (hER $\alpha$ ) transactivation assay was performed. Both pure *m,p'*- and *p,p'*-BGF displayed a significantly reduced oestrogenic potency (~426–457 times lower affinity than BPA) and oestrogenic efficacy (~39–50% of BPA's maximum induction). Interestingly, mutual comparison between *p,p'*-BPF and *p,p'*-BGF reveals and proves for the first time the direct link between *ortho*-methoxy substitution and reduced *in vitro* oestrogenic activity (for transactivation of hER $\alpha$ ). In contrast to *o,p'*-BPA, *viz.* the main byproduct of *p,p'*-BPA synthesis, *m,p'*-BGF was proven suitable for utilization in thermoplastics, thereby avoiding time-consuming and labour-intensive (re)crystallizations to obtain pure *p,p'*-BGF.

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## Plasmon-embedded TiO<sub>2</sub> thin films for efficient self-cleaning coatings

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Plasmon-embedded titania coatings show promising results in the field of self-cleaning materials. The embedded gold nanoparticles enhance the coatings' efficiency under solar light irradiation by extending the photoresponse to the visible light, which goes beyond the current commercial titania coatings, since they can only absorb UV-light [1]. A new synthesis method (with a patent pending PCT/EP2018/079983) using a one pot titania sol-gel with suspended gold nanoparticles was developed and thoroughly characterised. To determine the effect and location of the nanoparticles, the used characterisation techniques included absorption spectra analysis, film thickness and transparency measurements, AFM, XRD, SEM, EDX, TEM and contact angle measurements. The self-cleaning activity of the coating was evaluated using the established stearic acid degradation test using both UV light and a solar simulator and ISO 27448: 2009. The newly developed coatings with up to 3 w% Au nanoparticles to TiO<sub>2</sub> proved to be up to 16% and 40% more efficient than the pure titania coating under UVA and simulated solar light respectively. The newly developed coatings also showed an order of magnitude higher efficiency than a similar sol-gel-based coating with embedded gold nanoparticles developed by Sonawane *et al.* [2].

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## Stable plasmonic enhancement in photoelectrochemical cells for air purification with simultaneous H<sub>2</sub> production

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A new all-gas-phase photoelectrochemical cell enables the oxidation of hazardous organic pollutants at a TiO<sub>2</sub> photoanode, while simultaneously renewable H<sub>2</sub> is collected at the dark cathode [1]. Unfortunately, TiO<sub>2</sub> is only activated by UV light (< 5% of sunlight on earth). To broaden the activity window, the use of plasmonic ‘rainbow’ catalysts is studied [2]. Here, three TiO<sub>2</sub> species (Anatase, PC500 and P25) were photo-impregnated with 0, 1 and 2 wt% of spherical plasmonic Au-Ag nanoparticles, that effectively absorb visible light. An activity screening for the degradation of stearic acid under simulated sunlight was performed to find the most active composite. P25+2wt% outperformed the pristine P25 (+52%) and P25+1wt% (+22%). We showed it could be produced in a cost-effective way. P25 also outperformed PC500 (+31%) and pure anatase (+160%).

A major issue remains the instability of the plasmonic metal nanoparticles on the long term. Stable core-shell metal particles were therefore prepared by applying a Layer-by-Layer method [3]. Hereby, positively and negatively charged polyelectrolytes were used to create a very controllable protective shell around the particles. The success of this strategy was demonstrated by a salt addition test, that destroyed the bare particles while the protected nanostructures remained unaffected. Furthermore, it was shown that the activity of the stabilised plasmonic photocatalysts did not decrease significantly.

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## Development of a sustainable method for the extraction of suberin-related compounds from biomass using supercritical transesterification (SCTE)

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Suberin is a biopolyester which has a unique molecular diversity, through a variety of long-chain aliphatic molecules such as  $\omega$ -hydroxyacids,  $\alpha,\omega$ -dicarboxylic acids and their functionalized derivatives. However, the use of suberin as a source of building blocks on an industrial scale for the development of new products and materials is hampered by the chemical resilience of suberin. Of all the methods that have been developed to depolymerize and extract suberin, transesterification is one of the most widely used. But it has the disadvantages of requiring a catalyst, having lower yields in the presence of water and free fatty acids, and requiring long purification steps afterwards.

This work is based on the development of a SCTE method that offers the advantages of being tolerant to the presence of water and free fatty acids while being catalyst-free<sup>2</sup>. As a result, the SCTE method has made it possible to extract suberin from potato peels, cassava peels and oak bark in only 1 hour with good yields and high selectivity, thus limiting the need for purification. The results also show that by adjusting the treatment duration, it is possible to obtain a total or partial depolymerization of the suberin depending on the desired end product. SCTE is therefore a promising tool for extracting monomeric suberin that could be used to produce polyurethane foam and bactericidal films for surface treatment.

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## Characterization of pyomelanin pigment produced by *Y. lipolytica* and its application in green synthesis of gold nanoparticles

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Melanin is an heterogeneous group of polymers produced by several organisms through different biochemical pathways. Based on their biosynthesis pathway, they have been classified into three groups: eumelanins (black or brown pigments), pheomelanins (yellow-red pigments) and allomelanins that includes different pigments such as DHN-melanins (grey-green pigments) and pyomelanins (brown pigments). Melanin pigments confer to cells a high protection against different environmental stresses, principally UV radiations, oxidative stress and toxic effect of heavy metals.

In this study, we report on the ability of the *Y. lipolytica* strain W29 to produce melanin pigment at the yield of 0.5 mg/mL. The pigment has been identified as pyomelanin and its synthesis was found to occur from tyrosine by the so-called HGA-melanin pathway.

Based on its redox proprieties and its affinity for metals ions, the purified pyomelanin was used for the green synthesis of gold nanoparticles (AuNPs). The synthesized AuNPs with median size value of 104 nm were found of nanocrystalline structure, mostly polygonal or spherical. They exhibit high colloidal stability with zeta potential of -28.96 mV and moderate polydispersity index of 0.267. Moreover, the synthesized AuNPs were also characterized as non-cytotoxic toward two mammalian cell lines, namely the mouse fibroblast NIH3T3 and human keratinocytes HaCaT. Cell viability was only reduced at AuNPs concentration higher than 160 µg/mL. These results suggest that the green synthesized AuNPs could provide a powerful tool for imaging and diagnosing in medical field.


**Keywords:** pyomelanin, *Y. lipolytica*; HGA-melanin pathway; gold nanoparticles; mouse fibroblasts; human keratinocytes

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